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From the Departments of Dermatology (Head Nils Thyresson, M D) and Medicine (Head Erik Ask Upmark, M D) University Hospital Uppsala Sweden

Basophil Leukocytes in Cantharidin Blisters on Patients with Internal Disorders

By

LENNART JUHLIN and BENGT WADMAN

An increased percentage of basophils in artificially produced skin blisters was first described by Strauss in 1898 on patients with myelogenous leukemia (20) His results were confirmed the following year by Milchner (14) Some years later Klausner and Kreibich recorded an increase of basophils in vesicles produced on the cutaneous tuberculin reactions of Moro (11, 12) They also described a patient with vesiculous contact dermatitis, whose blisters, according to a picture, contained about 30 per cent basophils 66 per cent eosinophils and a few lymphocytes Their findings were partly confirmed by Pulitz in 1912, who also called attention to the simultaneous occurrence of eosinophils (16) These works seem then to have been forgotten although some are cited by Michels in his classic work on the basophil (13)

More extensive studies of the basophil in inflammatory exudates were first made by Rebuck et al 1960 and 1963 (15, 17) Using the skin window method they found an increase of basophils in the exudates of the patients with ulcerative colitis interstitial cystitis and Hurler's disease Their results gave us the incentive to study the basophil leukocyte in exudates of blisters produced by cantharidin in the skin of patients with various disorders An increased percentage of basophils was first found in patients with erythroderma and actinic dermatitis (6) From studies on dinitrochlorobenzene sensitized patients contact dermatitis and positive patch tests it seems now well established that the basophil percentage is increased in allergic inflammatory reactions of delayed type but not in toxic reactions (1 2 3 8 10 23)

In patients with allergic skin disorders, the increased percentage of the basophils in blisters could be found on normal appearing skin (8, 10) It would therefore seem possible that patients with some allergic internal disorders might also react with an increased leakage of basophil leukocytes

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Fig 1 Basophil leukocytes in cantharidin blisters

If such an increased leakage can be taken as a sign of an allergic reaction of delayed type, it might also be a helpful means for obtaining information on the etiology of diseases We have therefore studied the basophils in cantharidin blisters produced on patients with various internal diseases

Methods

Patients The diagnosis and numbers of the patients appear in fig 1 and tables II—III Patients with internal disorders mentioned in an earlier paper (8) are included in the material Some cases are further described under *Results*

Eliciting of inflammatory exudate An inflammatory blister was produced on the inside of the forearm by applying 0 5 per cent cantharidin for 20 hours It was applied under an adhesive bandage as described previously (8) When removing the bandage care was taken not to destroy the blister formed

Examination of basophils and eosinophils The whole content of the blister (0 05—0 4 ml) was aspirated with a siliconized needle and syringe, then forcibly ejected into 10 ml of a fixative with the following composition chloroform 10 acetic acid 30 and ethyl alcohol 60 The cells were concentrated on a Cella 0 filter and stained for 40 seconds with 0 02 per cent eosin (Gurr) in 70 per cent ethyl alcohol followed by 0 3 per cent toluidine blue at pH 5 2 for 30 seconds The filters were than dehydrated, cleared, and mounted as described earlier (8) The percentage of basophils and eosinophils was estimated by counting the number of white cells while finding 20—40 basophils The number of white cells seen varied between 2 000 and 5 000 In the beginning of this study the basophils were stained only with toluidine blue as earlier described (8)

Results

Controls 40 subjects, ages 21—51

The percentages of basophil leukocytes in the exudates are given in fig 1 They did not exceed 0 9 per cent in any of the individuals In the beginning of our studies the filters were stained only for basophils, the eosinophils therefore were counted in only 15 of the subjects They varied between 0 and 2 per cent

Eosinophil leukocytes in all patients An increased percentage (> 2 per cent) of eosinophils in the blisters was only seen in some patients with asthma who also had an increase of eosinophils in the blood

Alcoholism 21 subjects, ages 28—82

All the patients studied had been alcoholic addicts for more than two years the majority ten years or more

TABLE I Data on patients observed for hyperparathyroidism

Age	Sex	History of renal calculi	History of peptic ulcer	Result of surgical exploration	Serum			Urinary excretion of calcium (mEq/24 hrs)	Tubular reabsorption of phosphate (per cent)	Basophils in blisters (per cent)
					Calcium (mEq/L)	Magnesium (mEq/L)	Phosphate phosphorus (mg/100 ml)			
64	♀	−	−	Adenoma	5 6−5 8	−	2 1−2 4	17−23		1 8
77	♀	−	+	Adenoma	5 6−6 8	−	1 8−2 0	3− 5	79−93	1 5
32	♂	+	+	Adenoma	5 5−6 0	−		20−30		1 1
73	♀	+	?	Adenoma	6 2−7 8	2 1	1 7−2 8	5−11	62−80	0 5
49	♀	+	−	Adenoma	5 0−7 1	−	2 6−3 5	15−27	66	0 4
51	♀	+	−	Adenoma	5 7−6 1	2 0	2 8−3 1	5−15	79−89	0 05
47	♂	+	?	Adenoma	5 0 -5 6	1 9	2 6−2 9	17−19	87−90	0 05
55	♀	−	−		4 9−5 7	1 6	2 4−3 5	7− 8	88−92	3 2
18	♂	−	−		5 1−5 3	−	1 9−4 3	9−15	95−99	2 0
50	o	+	−		4 6−4 9	−	2 8−3 9	10−15	90−94	2 0
48	♂	+			4 6−5 1	−	2 2−4 2	16−20	94	1 2
83	♀	−	−		5 8−6 6	1 9	1 9−2 3	8−12	75−89	1 0
69	♀	−	−	Negative	5 9−8 2	1 9	2 0−2 3	8−21	74−78	0 6
47	♀	−	−		5 1−5 5	1 9	4 0−5 4	10−13	87−88	0 4
66	♂	+	+		5 0−5 2	1 7	2 8−6 5	9−10	82−91	0 3
25	o	?	−		4 6−5 6	1 8	2 8−3 9	9−16	94−97	< 0 1
58	♀	+	−		4 9−5 4	2 0	2 8−3 9	7−11	87−93	< 0 1
39	♂	+	−		4 7−5 2	1 8	2 0−3 0	13−17	96−97	< 0 1
26	♂	+	−		4 7−5 5	2 1	2 8−3 9	15−17	92−95	< 0 1
59	♂	+	+		4 5−5 4	−	2 2−3 1	18−19	84−92	< 0 1
Normal values					4 2−5 2	1 9−2 4	2 3−4 4	<15	86−95	< 0 9

When the studies were made the patients had been hospitalized from 2 days to 2 months Serum transaminases (SGOT and SGPT) were determined in 15 cases and found slightly elevated in eight cases four of these eight had a normal basophil count Needle biopsy of the liver was performed in seven of the cases Two of these showed fatty infiltration and 0 9 and 1 9 per cent basophils in the blisters, and five had Laennec's cirrhosis with normal basophil counts

Polycythemia 9 subjects, ages 24—76
In four cases the diagnosis was polycythemia vera two of them had more than 1 3 per cent basophils in the blisters and about the same per cent in blood Both of them had moderately elevated numbers of leukocytes (12—16 000 per mm³) and thrombocytes (430—810 000 per mm³) in the blood The other two cases had 0 7 and 0 3 per cent basophils in the blisters One of them had leukocytosis of the blood but no thrombocy-

tosis at the time of the study The other one was successfully treated with P^{32} four years ago and had normal counts of leukocytes and thrombocytes In the table are included five cases of polycythemia which was regarded as secondary to other diseases, e g diseases of the cardiovascular system Only one of these had an increased basophil percentage in his blister This patient probably was or had been an alcoholic addict There was no case of thrombocytosis or leukocytosis in this group

Chronic myelocytic leukemia 7 subjects, ages 46—79

In six cases the diagnosis was substantiated by sternal puncture findings and/or autopsy The duration of the disease varied between one and ten years Three patients had an elevated percentage of basophils in their blisters 1 5, 1 2, and 1 0 and their correponding percentage of blood basophils was 5 0, 12 5, and 7 8 respectively The three patients with a normal percentage of basophils in their blisters had 0 5 2 5, and 3 per cent basophils in blood The seventh case in the table was hospitalized for the first time only a few weeks before the test was made She had 7 6 per cent basophils in her blister but only 2 2 in the blood There was also a moderate thrombocytosis of 7—800,000 per mm³ and in a marrow biopsy a prominent hyperplasia of megakaryocytes The number of leukocytes was about 30,000 with 2 per cent myelocytes and 1 5 per cent metamyelocytes The hematocrit was 48—50 per cent We cannot exclude the possibility of this patient being in an early phase of a myeloid metaplasia or a polycythemia vera

Chronic lymphocytic leukemia 7 subjects, ages 38—76

The diagnosis was histologically beyond dispute in six cases of long duration (2—11 years) In one case of short duration, the pathologist cannot exclude a lymphosarcoma This patient had 2 3 per cent basophils in the blister Differential blood count showed 1 per cent basophils The three patients with a normal percentage of basophils in their blisters were all treated with prednisolone or related steroids

Thrombocytopenia 7 subjects, ages 42—66

All patients had the thrombocytopenia as a dominant symptom, there were no signs of leukemia One patient with 1 1 per cent basophils in his blister had a splenectomy for hemolytic anemia about ten years before this study

Rheumatoid arthritis 16 subjects, ages 28—77

Of the four cases with an increased number of blister basophils, one was probably developing sclerodermia out of a long standing rheumatoid arthritis, one had a peculiar variety of polychondritis affecting primarily his ears, and one had no arthritic symptoms at all at the time of the investigation, but noticed his first arthralgia about two months afterwards Two of them had strongly positive serological tests for rheumatoid arthritis, both were treated with moderate doses of predni

TABLE II Various diseases showing an increased number of basophils

No of patients	Diagnosis	Basophils in blisters (per cent)
1	Klinefelter s syndrome	1 3
3	Hypopituitarism	0 0 3 2 5
1	Brain tumor (hypothalamus)	1 7
1	Hypogonadism	2 3
3	Acute nephritis	0 1 1 1 2 2
3	Myocarditis (nonrheumatic)	0 0 8 1 0
3	Ankylosing spondylitis	0 0 2 5
1	Polychondritis	1 9
7	Sarcoidosis	0—0 3 1 2 1 4
1	Myxoma of left atrium	1 3
2	Hypernephroma	0 2 2 7
1	Leukemoid reaction	4 6

solone Among the twelve patients with normal basophil percentage, serological tests were performed in six cases The reaction was strongly positive in one case, uncertain in another, and in four cases was negative

Ulcerative colitis 32 subjects ages 16—82
Two patients had a blister basophil percentage of more than 2 per cent In one of them a total colectomy was performed 2 years before the present investigation He still had, however, anemia and an accelerated ESR The other patient with over 2 per cent basophils had had symptoms of his disease for seven years Their percentages of blood basophils were normal Most of the patients examined were treated with salicylazosulfapyridin No case was in a state of acute bleeding at the time when the blisters were induced

Hyperparathyroidism and other hypercalcemic or hypercalcuric disorders 20 subjects, ages 18—23
The patients were hospitalized for recidivating nephrolithiasis and/or accidentally found hypercalcemia Surgical exploration was done in eight cases and a parathyroid adenoma was found in seven of them (table II) In the other cases indications for surgical exploration of the parathyroid gland at the time of investigation was not yet considered necessary No other explanation for the abnormal calcium metabolism could be supported in any of these cases The patient without adenoma had been thyroidectomized 5 years earlier and treated with thyroid hormone Two patients with 0 5 and 3 2 per cent basophils were also treated with thyroid hormone for myxedema The patient with 1 5 per cent basophils had a minor leg ulcer without any surrounding eczema A history of peptic ulcer was

TABLE III Diseases with a normal basophil percentage in the blisters (<0 9%)

Diagnosis	No of pat
Anemia and gastointestinal diseases	
Hypochromic anemia of iron deficiency	14
Malabsorption syndromes	4
Peptic ulcer	9
Regional enterit s (Crohn)	2
Non-specific colitis	3
Internal fistula of the intestine	1
Malignant disorders	
Adenocarcinoma	5
Acute leukemia	5
Multiple myeloma	2
Hodgkin s disease and lymphosarcoma	10
Allergic and metabolic disturbances	
Bronchial asthma	9
Intermittent hydrarthrosis	2
Gout	1
Nephrotic syndrome	3
Reiter s disease	2
Hormonal disturbances	
Myxedema	3
Acromegaly	1
Diabetes mellitus	5
Cardiovascular system	
Congestive heart failure	5
Multiple arterial stenosis of unknown etiology	6
Giant cell arteritis	1
Various collagen diseases (systemic lupus erythematosus scleroderma)	6
Acute myocardial infarction	10
Malignant hypertension	1
Diseases of the liver	
Cirrhosis	11
Acute hepatitis	1
Congestion of the liver	5
Various	
Non specific conditions with increased ESR	10
Patients admitted to the hospital for investigation of conditions proved to be non pathological (for example physiological heart murmurs)	8

recorded in four of the cases as shown in table II, where the ranges of the blood and urine values for calcium, magnesium and phosphate are also given No correlation was found between these values and the basophil percentages in their blisters

Pernicious anemia 12 subjects, ages 59—85

All patients had achylia and serum B 12 values below 100 $\mu\mu$g/ml, most of them 40 $\mu\mu$g or less Their hemoglobin was between 6 and 10 g % Treatment with vitamin B 12 was started at the same time or some days before the blister was produced One case had a deficiency in folic acid as well The patient with 4 per cent basophils in his blisters was a 65 year old man who had a total gastrectomy for a bleeding ulcer 10 years earlier In this respect he differed from the others and ought, therefore, to be classed only as megaloblastic macrocytic anemia

Various diseases with a basophil increase (> 0 9 %)

The disorders in which an increased percentage of basophils in blisters was

found are listed in table II The following comments should be made

The single case of Klinefelter's syndrome had a female chromatin pattern, hypogonadism, and also a patent ductus arteriosus The other case of hypogonadism probably had a chromosomal aberration as well, but not a female chromatin pattern

The case of acute nephritis with 0 1 per cent basophils was a young woman with proteinuria and accelerated ESR but without elevation of antistreptolysin titer, therefore, proteinuria might be a more correct diagnosis

The three patients diagnosed as myocarditis were young men with ECG signs of myocardial damage and/or pericarditis Etiologically they were classed as viral infections without any signs of a rheumatic process of streptococcal origin

Diseases with a normal basophil percentage
The disorders in which a normal percentage of basophils in blisters was found are listed in table III

Discussion

In inflammatory exudates produced in the skin on patients with internal disorders an increased percentage of basophil leukocytes has earlier been reported in ulcerative colitis, leukemia in some single cases with peptic ulcer rheumatoid arthritis, myelofibrosclerosis and pernicious anemia (8 14 17 20) These, as well as those disorders which during our study also showed an increased leakage of basophils were therefore studied in detail

Why the basophils are increased in exudates of patients with certain disorders is hitherto unknown When the basophils are increased in the blood a direct leakage could be an explanation Blood basophilia has been reported in patients with polycythemia and chronic myelocytic leukemia It would, therefore, seem likely that the basophils in such cases also are increased in the inflammatory exudates as was reported in the beginning of this century (14 20) However, in a patient with 37 per cent basophils in the blood Rebuck et al found *the same sparse migration* of basophils in the exudate as in control subjects (15 17) Riis described four cases of myeloid leukemia where the percentages of blood basophils were high (3—15 per cent) and where the exudates in skin window preparations only occasionally showed 4 per cent basophils (18) None of his leukemic patients with normal blood basophil counts had a high percentage of basophils in exudates In our patients with chronic myelocytic leukemia and polycythemia, an increase of blister basophils was found in patients who also had an increase of basophils in the blood That these results differ from those of Rebuck et al is probably due to the cantharidin used by us In these two disorders the basophils increase might therefore be explained by a direct nonspecific leakage of the cells from the blood vessels Specific basophilotactic factors cannot however be excluded since patients with chronic lymphatic leukemia without blood basophilia also had an increase of basophils in their blisters In this connection we want to

point out that we found no basophilia in the blood in the other diseases studied, and in earlier studies no correlation between the percentage of blood and exudate basophils has been shown (8)

Rebuck et al found that twenty-six of forty one patients with ulcerative colitis had an increase of basophils in the exudate of skin window preparations (17) In earlier studies we found a less frequent increase (7) We have, therefore, studied the leakage of basophils in additional patients, but found a basophil increase in only six of thirty-two cases The cause of the discrepancy is probably due to the differences in methods used and has been discussed elsewhere (8) The problem has recently been investigated by Wolf Jurgensen et al (24) In eleven patients with ulcerative colitis and thirteen with other diseases, they studied the leakage of basophils into an inflammatory exudate with the skin window method described by Rebuck et al This technique includes the application of diphtheria toxoid to obtain an exudate They found the basophil increase only in Schick negative patients and considered the basophil increase to reflect a delayed hypersensitivity to diphtheria toxoid Their findings might explain the discrepancy between Rebuck's and our result, but do not give any clue to the basophil increase found in six of our patients with ulcerative colitis

An increase of basophil leukocytes has earlier been demonstrated in exudates of patients with contact dermatitis (1, 2, 3, 8, 10) and also in dermatoses where a delayed type of allergic reaction is probable (3, 8, 10) In toxic reactions no basophil response was obtained (1, 2, 8) That the basophil increase was inhibited by local pretreatment with a corticosteroid favors an allergic pattern of reaction (9) It is, however, uncertain if the basophil increase can always be taken as a sign of delayed type of allergy since it also occurred in some dermatoses of unknown origin, for example psoriasis and alopecia areata (10)

In skin diseases the basophil increase was found both on normal appearing and affected skin Here one could assume that cantharidin initiated an allergic reaction, for example by liberating a more or less incomplete antigen antibody complex Such a hypothetical complex might have basophilotactic properties which became more efficient when the hapten or antigen was added It is, however, more difficult to explain tha basophil increase in internal diseases In most of the disorders where a basophil increase in the exudates has been found autoimmunity has been discussed as a possible etiological factor (for ref see Waksman (21) and articles in monographs by Gell and Coombs (4) and in N Y Acad Sci (22)) Not all of the autoimmune reactions however, are of the delayed type, for example systemic lupus erythematosus, which can explain why we have found no such patients with a basophil increase The same holds for asthma which belongs to the immediate type of reaction Thus, it is not possible to exclude that a delayed type of allergy is involved in the disorders showing a basophil increase Support for such a view has recently been given by Wolf Jürgensen and Halberg who reported an increase of

basophils in skin windows of patients with Hashimoto s thyroiditis after application of thyroid extract (25)

The only group of patients where an allergic etiology is rarely mentioned is that of alcoholic addicts Since many of our alcoholics studied showed signs of liver damage this could be a factor which in some way initiated their basophil increase We extended therefore our studies to include milder forms of alcoholism in patients treated in the psychiatric ward With the cooperation of Dr Gudrun Wadman another group of thirty patients was studied In six of them the serum transaminases GOT and/or GPT were slightly increased and among them we found three who showed an increase of basophils in the blisters All other patients had normal basophils The basophil increase in alcoholics might therefore be secondary to some hepatocellular injury Against such a hypothesis stands the finding that none of the five alcoholics with Laenec s cirrhosis or the seventeen patients with diseases of the liver showed a basophil increase Further studies are therefore needed to ascertain if alcoholics with a basophil increase are of a special type and have other characteristics which makes them react differently

Another group of patients which we have studied with special interest is that with hyperparathyroidism The relationship between calcium and mast cells has been reviewed by Selye (19) He has formed the concept of calciophylaxis, i e calcification of the skin can be produced where a histamine liberator is injected in animals sensitized with parathyroid hormones and injected intravenously with for example $FeCl_3$ a topical Calcium is bound by the released mast cell granules One could therefore speculate that the leakage of basophils in a similar fashion could take part in the formation of calcium deposits as for example in renal calculi

We have not been able to correlate the number of basophils with any available chemical laboratory data A lowered level of magnesium was found in fifteen of thirty five patients with the disorders listed in fig 1 but there was no correlation between the basophil percentage and the amount of magnesium It is interesting in this connection to note that in the acute phase of magnesium deficiency in rats, a degranulation of mast cells and both blood and tissue eosinophilia has been found which are often used as signs of allergic reactions (5)

Summary

An increase of basophil leukocytes has earlier been found in allergic skin reactions of delayed type In patients with allergic dermatoses the basophil increase could also be seen in inflammatory exudates produced with cantharidin ointment on normal appearing skin We have here studied the basophils in such lesions produced in 289 patients with internal disorders and 40 healthy subjects In the healthy subjects the exudates never contained more than 0 9 per cent basophils and 2 per cent eosinophils These values were therefore chosen as the upper limits of a normal response An increase of eosinophils was only found in some patients with

asthma who also had eosinophilia in the blood The basophils were increased in blisters in 40 per cent of those with signs of hyperparathyroidism and 48 per cent of the cases with alcoholism In ulcerative colitis and rheumatoid arthritis an increase of basophils was seen in 19 respectively 25 per cent of the cases A basophil increase in the blisters was often found in patients with thrombocytopenia, chronic lymphocytic leukemia, chronic myelocytic leukemia, and polycytemia In the two last mentioned disorders, the basophils were also increased in the blood, and the blister basophilia could be explained by a direct leakage from the blood In the other disorders with a basophil increase, there is no correlation with the basophil percentage in blood

An increased percentage of basophils in exudate has also been found in some patients with the following clinical entities pernicious anemia, Klinefelter's syndrome, hypopituitarism, tumor of hypothalamus, hypogonadism, acute nephritis, myocarditis, ankylosing spondylitis, polychondritis, sarcoidosis, myxoma, hypernephroma, and leukemoid reaction

We have not been able to correlate the number of basophils with any available chemical laboratory data The possibility that the basophil increase might signify an allergic etiology of delayed type has been discussed

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From the Renal Ward Fourth Medical Clinic University of Helsinki, and the Finnish Red Cross Blood Transfusion Service Helsinki Finland

Acute Renal Failure after Haemolysis, Probably Due to Foeto-maternal Transfusion

By

A PASTERNACK U FURUHJELM, J VON KNORRING B SKRIFVARS and B KUHLBACK

Intravascular haemolysis with subsequent acute renal failure is usually due to incompatible transfusion or to toxic or infectious conditions A case is reported where haemolysis leading to acute renal failure probably developed after transfusion of incompatible blood from foetus to mother

Case report

The patient a 22 year-old primigravida at the end of the eighth month was admitted to the Renal Ward through an obstetric clinic because of acute renal failure A week earlier she had been vomiting and on the day of admission had complained of severe headache and abdominal pain and had convulsions At that time foetal heart sounds had been heard by an obstetrician Physical examination on admission June 28 1965 revealed an unconscious patient with frequent convulsions and a moderate hypertension of 170/120 mm Hg No foetal heart sounds were audible The patient passed 150 ml of brownish urine which contained much haemoglobin Her serum was red-coloured and contained free haemoglobin as shown by spectrophotometric analysis and bilirubin of the indirect type (icterus index 1 30) The blood haemoglobin level was 12 0 g per cent the erythrocyte count 3 840 000 per mm³ and the platelet count 65 000 per mm³ The reticulocyte count was 1 1 % The serum contained 10 mg per cent of free haptoglobin Serum iron was 333 per cent After the first day no further haemolysis occurred as judged by the absence of free haemoglobin and bilirubin in the serum There were no signs of severe infection (blood culture negative)

On the second day the patient gave birth to a dead normally developed male foetus weighing 1,220 g Autopsy was not performed The placenta was considered normal During the first two days in hospital the urinary output was 700 ml per day Plasma creatinine rose to 3 75 mg per cent After two days recovery took place with an ordinary diuretic phase and subsequent restoration of renal function

After the first two days no further convulsions occurred the patient became conscious on the fourth day the blood pressure was normal and the platelet count became normal The haemoglobin level dropped slowly being 8 0 g per cent one week after admission Concomitantly with the decline of the haemoglobin there was a rise in the reticulocyte count the peak of 6 5 % being reached two weeks after the haemolytic episode The patient never had hepatomegaly

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TABLE I The patient s isoagglutinins isohaemolysins and immune isoagglutinins after mercapto-ethanol treatment (3)

	27 7 65	9 9 65	21 10 65
Anti A agglutinins	1 128	1 64	1 32
Anti A haemolysins	1 1	1 1	0
Anti B agglutinins	1 32	1 32	1 8
Anti B haemolysins	—	—	—
Anti A immune agglutinins		1 1 strong	1 1 very weak
Anti B immune agglutinins		—	—

but on admission the activity of glutamic oxalacetic transaminase in the serum increased to 320 units indicating liver damage One week later these values had returned to normal

Renal biopsy, performed on *July 12 1965* showed changes consistent with acute tubular necrosis in regeneration No glomerular or vascular changes were observed

Serological tests The patient belonged to blood group O Rh positive her husband to group A Rh positive The direct Coombs test was negative Tests for cold agglutinins were negative The patient s isoagglutinins were titrated one, two and a half and four months after the haemolytic event The results are shown in table I

Discussion

It seems probable that the acute renal failure in the case reported was related to severe intravascular haemolysis The haemolysis was of short duration and apparently did not affect the patient s haemoglobin level The reticulocytosis developed slowly and was probably not related to the haemolysis

A toxic or infectious aetiology of the haemolysis can be ruled out by the absence of these factors in the history Among other possible causes of haemolysis, an autoimmune aetiology would have led to a positive Coombs test (1)

The simultaneous occurrence of haemolysis, thrombocytopenia, central nervous symptoms and renal failure could be a manifestation of thrombotic thrombocytopenic purpura (5) However, the negative history of visible purpura, the rapid disappearance of the symptoms and the absence of microangiopathic lesions in the biopsied kidney do not support this diagnosis

An increasing number of cases have recently been described with a syndrome much like that of thrombotic thrombocytopenic purpura This syndrome has been called the haemolytic-uraemic syndrome (5 6) The present case, with its various symptoms, fits well with this disease entity However, the patient is an adult in contrast to all formerly reported patients who have been infants or children (4)

The fact that our patient was of blood group 0 and her husband of group A, suggests the possibility of incompatibility between the patient and her foetus Transplacental foeto-maternal transfusion with subsequent haemolysis of the

foetal red cells in the mother s circulation is thus a possible aetiological factor This pathogenesis is supported by the absence of anaemia in spite of haemolysis, and by the decreasing titres of both anti A isohaemolysins and anti A immune isoagglutinins Transfusion of incompatible blood could further be the cause of the thrombocytopenia which occurred in the patient (2) It seems likely that in this case the transfusion of incompatible blood was the cause of a hypersensitivity reaction like that thought to operate in the haemolytic uraemic syndrome (6, 7) leading to transient convulsions, hypertension and liver damage

Summary

Severe intravascular haemolysis of short duration occurred in a pregnant woman The haemolysis was followed by acute renal failure Transplacental foeto maternal transfusion of incompatible blood is thought to have been the pathogenetic mechanism This theory is supported by the absence of anaemia and reticulocytosis at the moment of haemolysis and by the presence of isohaemolysins and immune isoagglutinins in decreasing titres

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From the Department of Clinical Physiology and the First Medical Service Sahlgren's Hospital, University of Goteborg Goteborg Sweden

Atrial Flutter and Maximal Exercise

A Case Studied Before and After Conversion to Sinus Rhythm

By

GUNNAR GRIMBY, MAGNUS KORSGREN and NILS JOHAN NILSSON

The hemodynamic importance of atrial systole for the function of the heart is a problem that has recently attracted attention (4 9) Hemodynamic studies before and after conversion of arrhyth mia have led to a fairly general accept ance of the concept that the effective ness of the heart is improved in sinus rhythm, an improvement which is ac centuated during work (5 6) Explana tions for the better function in sinus rhythm have been sought in the more adequate adaptation of ventricular rate, the direct effect of the normal mechanical atrial systole, and the indirect effect of the atrial contraction on the closure of the mitral valve (11) However deter minations of hemodynamic alterations following conversion of atrial arrhythmia to normal regular rhythm at rest and in submaximal exercise, permit only an incomplete evaluation of the role of the atrial systole in man It has therefore been suggested that the changes during maximal exercise would constitute a more sensitive test of the importance of atrial contraction (4) Taking these aspects into consideration, we have regarded it worthwhile to report in detail a case which may contribute to the knowledge of this subject — We recently had the opportunity to investigate a well trained man, first in atrial flutter and later in sinus rhythm after treatment with DC countershock On both occasions the investigations included observations at his maximal level of muscular exercise The case was furthermore suitable for such a study because there was a high degree of block at rest, which decreased rather gradually during increasing work load Therefore the difference in ventricular rate at the same degree of work load before and after conversion was not as great as is often the case There was no other therapy instituted than the DC shock

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Case report

The patient was a 56-year old bus driver (71 kg 180 cm) who had been in good health except for a transient throat infection

TABLE I Circulatory data at rest and during exercise with atrial flutter and sinus rhythm

Rhythm	Work load (kpm/min)	Ventricular rate	Oxygen uptake (l/min)	Cardiac output (l/min)
Atrial flutter	Rest supine	43	0 23	6 0
Oct 23	Rest, sitting	50	0 36	4 5
	100	57	0 65	6 3
	300	58	1 05	7 7
	900 5 min	113	1 92	14 1
	7 min	147		16 7
	1,200	168	2 70	17 9
	1 500 1 5 min	194		
Sinus rhythm	Rest supine	53	0 30	4 5
Dec 14	Rest sitting	52	0 34	4 4
	100	61	0 64	6 2
	300	72	0 92	8 3
	900	117	2 01	13 5
	1,200	151	2 88	18 5
	1 500 2 5 min	153		19 0
	4 min	163		19 7

The values for ventricular rate cardiac output stroke volume and brachial artery pressure are the 1 500 kpm/min in sinus rhythm where the values are given separately

for a 3 week period in 1952 In September 1963 he had another throat infection with the suspicion of an incipient abscess which was adequately treated with penicillin He was completely recovered in 3 weeks

Since his early youth he had practised an extensive programme of physical training He took part in competitions of cross-country running up to the time of the period of investigation The physical training during the last year consisted of running 1—1 5 hours about three times a week including short spells of maximal exertion

His atrial flutter was discovered in October 1964 when he volunteered for an investigation on the cardiovascular function of active middle aged athletes which one of us (G G) was performing A careful retrospective history yielded no clue to the duration of the flutter The patient had experienced a transient decrease in his physical capacity in September 1964 in connection with a possible upper respiratory infection It may be assumed that the arrythmia started either at this time or at the time of the throat infection one year ago On the other hand a very long standing flutter cannot be excluded

The patient was examined on several occasions in October and November 1964 and he had a constant unchanged auricular flutter with an AV block of 6 1 to 7 1 at rest Except for the atrial flutter there were no pathological ECG changes The atrial rate was about 290 per minute and the ventricular rate consequently about 40 to 45 per minute at rest Careful physical examination revealed no other abnormalities The patient had a normal chest roentgenogram Routine laboratory tests were normal including BMR serum protein bound iodine and cholesterol

Conversion of the atrial flutter was considered to be indicated The patient agreed to have his cardiovascular function more closely studied before as well as after the

Stroke volume (ml)	Art ven O_2 diff (ml/l)	Brachial artery pressure (mm Hg)			Lactic acid conc mM	Pyruvic acid conc mM
		S	D	M		
140	38	144	71	97	0 9	0 10
92	80	141	66	93	–	
112	103	158	74	101	–	
133	136	157	69	96	1 1	0 [illegible]8
125	126	221	78	113	3 7	0 [illegible]
114						
106	150	175	78	113	7 1	0 [illegible]
		186	86	122	8 7	–
85	67	131	68	95	1 3	0 09
85	77	132	75	100		–
102	103	140	75	106	1 1	0 07
116	111	149	70	102	1 2	0 08
115	149	185	87	131	3 2	0 16
123	156	202	93	138	6 3	0 21
124		212	101	154	9 5	0 21
119		202	104	143		

means of two (at rest three) determinations except at 900 kpm/min in atrial flutter and at

conversion The conversion was performed according to the procedure described earlier from this hospital (7) which includes treatment with Dicoumarol for three weeks prior to the conversion no other drugs were given Dicoumarol treatment was discontinued following the conversion to normal sinus rythm. Conversion was performed at once with an energy level of 100 watt seconds All procedures were performed in the out patient clinic

Methods

The cardiac output was determined with the dye-dilution technique using a specially developed cuvette densitometer (10) with Cardio-green as an indicator Polyethylene catheters were introduced percutaneously into the brachial arteries and into a cubital vein The blood drawn through the densitometer was reinfused Blood pressure was measured with an inductance manometer simultaneously with the determinations of the cardiac output The horizontal plane through the sternal insertion of the fourth rib was taken as a reference level for the pressure measurement in the sitting position For the determination of oxygen uptake expired air was collected in Douglas bags and analyzed with the Scholander method Lactic and pyruvic acid concentrations in arterial blood were determined with enzymatic spectrophotometric methods

The first examination was performed one month before and the second 3 weeks after the conversion which was done November 24 1964 The same experimental procedure was followed on both occasions After three determinations of cardiac output at rest in the supine position the patient moved to an electrically braked bicycle ergometer and the cardiac output was measured with the patient sitting at rest after 7 and 9 minutes. Physical exercise then began Cardiac output was determined 5 and 7 minutes after each

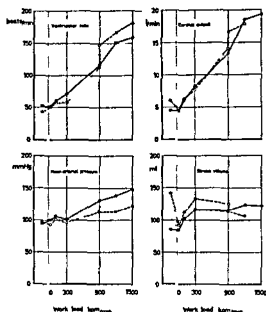


Fig 1 Hemodynamic data during rest and exercise in flutter (broken lines) and sinus rhythm (solid lines) The points to the left of the vertical dotted line represent the values at rest in the supine position

stepwise increase in work load, simultaneously with collection of the expired air and measurement of the arterial blood pressure

Results

The results are summarized in table I and in fig 1 During the investigation with flutter the atrial rate was constantly between 290 and 300 per minute Generally the values are the means of 2 (at rest 3) measurements At 900 kpm/min with atrial flutter, however the individual values are given for ventricular rate, cardiac output and stroke volume, as the AV block changed from mainly 3 1 to 2 1 between 5 and 7 minutes of work The patient stopped working because of exhaustion at 1,500 kpm/min, in the investigation with atrial flutter after 1 5 minutes and in the investigation with sinus rhythm after 4 minutes At the first examination, this short time did not permit any cardiac output determination, but at the second two determinations were performed

Two weeks after conversion to sinus rhythm, maximal oxygen uptake was determined according to Åstrand and Saltin (3) and found to be 3 1 liters per minute (43 ml/kg min) at a heart rate of 162 per minute The electrocardiogram was normal

Red cell volume determined with Cr^{51} was found to be 2 0 liters and total blood volume calculated to be 5 0 liters Hemoglobin was 15 4 g per 100 ml The heart volume in the prone position (8) was 760 ml with atrial flutter and 680 ml with sinus rhythm

In April 1965, five months after the conversion, the patient was again examined His ECG was still normal, and he could go on with the exercise test until 4 minutes on 1,500 kpm/min, i e his condition was exactly the same as 3 weeks after the conversion

Discussion

The treatment of this case of atrial flutter was successful, in that the sinus rhythm remained after conversion with DC countershock, without any maintenance therapy Since the patient had no complaints during the period of flutter, no other improvement from the clinical point of view could be demonstrated He reported however, a feeling of well being and a greater ease when performing maximal muscular exercise in his extensive training programme

The physical working capacity was already high in atrial flutter It may perhaps be regarded as somewhat higher after the conversion The possible improvement, however, could be discerned only by exhaustion on the highest work load setting in earlier in atrial flutter than in sinus rhythm

The cardiac output values during the *submaximal* part of the work tests were of the same magnitude in the two different states of rhythm as in the case reported by Åstrand et al (1) In relation to the oxygen uptake the cardiac output was of the same magnitude as found in normal young men (2) The stroke volume at rest and during light exercise was higher during flutter than in sinus rhythm The augmentation in cardiac output during atrial flutter from 100 to 300 kpm per minute was accomplished solely by an increase of the stroke volume

The ventricular rate during flutter, which on the lighter work loads was lower than with normal rhythm, increased so much in the middle work levels that it was considerably higher on the maximal loads This sudden increase in ventricular rate, which took place in the course of the exercise on 900 kpm/min was accompanied by a definite reduction of the stroke volume After this at *heavy and maximal* exercise, the stroke volume remained smaller in atrial flutter than in sinus rhythm Although we have no determination of cardiac output at the highest work level in atrial flutter the available determinations suggest that very nearly the same cardiac output could be reached as in sinus rhythm The early levelling off of the cardiac output values (see the table) at 1,500 kpm/min in sinus rhythm, as well as the high lactic acid concentration in this situation, indicate that those cardiac output values were maximal for the individual During rest and light to medium work loads there was a greater pulse amplitude in flutter than in sinus rhythm but the mean arterial pressure was lower

The maximal values of cardiac output and muscular work for this well trained, symptom free man are only fractionally lower during atrial flutter than in sinus rhythm the difference being so small as to be hardly noticeable The main differences are that in flutter the maximal cardiac output is maintained at a higher ventricular rate with a smaller stroke volume and at a lower arterial blood pressure This study thus does not give any convincing evidence for an important role of the atrial contraction as such in the attainment of even maximal cardiac output

Summary

A case of atrial flutter in a well trained symptom free 56 year old man is described Hemodynamic studies with determinations of cardiac output and oxygen consumption, in rest and in exercise including maximal work level, were performed before and after conversion with DC countershock, without any other therapy At submaximal exercise the cardiac output was of the same magnitude in atrial flutter and sinus rhythm The stroke volume was higher in atrial flutter at rest and light exercise but lower at heavy exercise The maxi

mal values for cardiac output and muscular work were only just noticeably lower during atrial flutter than in sinus rhythm The effect of the mechanical atrial systole in these respects therefore does not seem to be important

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From the First Department of Medicine (Head Pentti I Halonen M D) University of Helsinki Helsinki Finland

Insufficient Cardiorespiratory Response to Exercise Secondary to Central Nervous System Lesions

By

M H Frick Olli P Heinonen and J Heikkila

The growing interest in neurocardiovascular problems, especially concerning the role of the higher cardioregulatory centers, is reflected in two recent symposia (1, 11) The participation of medullary and supramedullary regions in cardiac control has been illustrated in animal experiments, but the clinical counterparts of the cardiac effects of experimental brain lesions are limited mainly to the sub endocardial changes evoked by intracranial bleedings (7 17) On the other hand, the circulatory effects of spinal cord lesions (13) diabetic neuropathy (14) and carotid sinus hypersensitivity (15) are well delineated

In 1964 we reported a patient whose heart rate in *sinus rhythm* could not be raised over a limit of 120/min by physical exercise of pharmacological agents (4) This patient had years ago had encephalitis and showed neurological sequelae at the time of the study Pressure and flow measurements revealed that the limited heart rate response to exercise was compensated by a 100 % increase in the stroke volume The peripheral vascular bed reacted normally on exercise and in postural blood pressure tests

The present report describes the second patient so far encountered with a similar heart rate restriction in *sinus rhythm* combined with normal peripheral vasomotion and neurological signs suggestive of a central origin of the syndrome

Case report

The patient was a 46-year-old farmer who entered the hospital June 3, 1965 for cardiological investigations The history revealed a head trauma with a brief period of unconsciousness in childhood As long as he could remember his mouth had been drawn out of shape but he had no engrams of a febrile illness in connection with the facial paresis He had been hearing poorly since the age of 20 Bad hearing was frequent among his relatives

Starting from time of the World War II he had been distressed by vague dizziness in

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connection with muscular exertion This symtom was sometimes accompanied by a fit of cramps He had noticed no gross pulse-rate alterations during these attacks On June 8, 1964 when going to micturate at night he suddenly lost consciousness Examinations in a municipal hospital revealed supraventricular tachycardia of 150/min combined with a left bundle branch block and some ventricular extrasystoles in the ECG The subsequent electrocardiograms showed a normal sinus rhythm of 60—80/min normal a v conduction and the left bundle branch block Leucocytes were up to 10 300 per mm³ but the serum transaminases remained normal He was treated as having a myocardial infarction and made full recovery After this event he had suffered also from chest pain in connection with physical exertion Coronary dilators and digitalis were prescribed and an oral diuretic was added later

In October 1964 he was admitted to another municipal hospital because of continued exertional angina, a feeling of pressure in the neck and brief periods of unconsciousness Epilepsy was suspected and the treatment was changed accordingly This resulted in worsening of his cardiac symptoms, and the medication with digitalis diuretics and coronary dilators was resumed

General and neurological findings The patient was of average build There was a paresis of the left facial nerve Nothing abnormal was found in the pupils deep tendon and abdominal reflexes The Babinski sign was negative on both sides No rales were audible in the lungs and the liver was in the costal margin No ankle edema Blood pressure was 150/90 supine and the pulse rate regular at 72/min The postural blood pressure and pulse reactions were tested several times with normal findings The right radial pulse disappeared when the right arm was raised over the head level Simultaneously a weak systolic murmur became audible on the right side of the neck A grade II/VI systolic ejection type murmur with no diagnostic characteristics was audible in the third and fourth left intercostal space near the sternal border The second sound in the pulmonary area was split The eye grounds showed changes of grade II of Keith and Wagener

The ECG revealed sinus rhythm with normal a v conduction, total left bundle branch block and no signs of the previous dubious myocardial infarction Long tracings revealed occasional atrial ectopic beats Chest X ray showed no pulmonary pathology and a heart of 435 ml/M² BSA with a slight left ventricular prominence X ray films from the skull were normal and the intervertebral foramens of the cervical spine were not narrowed Vital capacity and forced expiratory volume were 85 %, maximal breathing capacity and expiratory peak flow 90 % and 100 % respectively

In a detailed neurological examination at the Department of Neurology, an insufficient eye convergency was found in addition to the facial paresis of central origin The electroencephalogram was not diagnostic The ophthalmodynamometric tracings revealed symmetrical and normal systolic and diastolic pressures An audiogram was consistent with otosclerosis

Laboratory data showed a normal BSR no anemia, a normal amount and differentiation of leucocytes normal serum cholesterol and PBI normal serum creatinine no sugar, protein or formed elements in the urine normal blood sugar normal serum electrolytes and negative blood Wasserman tests, normal acid base balance at rest and during moderate exercise normal 24 hour excretions of catecholamines and vanillin mandelic acid normal antistreptolysin and antistaphylolysin titers, and negative rheumatoid factor LE-cell and toxoplasma dye tests The cerebrospinal fluid was under normal pressure with no cells normal protein concentration and negative Wasserman test Paper electrophoresis and immunophoresis of the serum proteins were normal

Cardiological investigations An aortography showed normal right sided subclavian caro-

t d and vertebral arteries The left carotid artery was also normal but there was a long relat ve occlus on of the subcla ian artery just after the bifurcat on of the internal mammarian artery (fig 1) the left vertebral artery ris ng from th s occlus on and being filled slowly in the cephalad direction There were no s gns of a retrograde filling of the subclavian artery via the vertebral artery

The exercise tolerance was tested by an electrically braked ergometer (Elema Schonander) the pat ent pedalling in the s tting pos t on The ECG was cont nuously mon tored through b polar electrodes attached to the chest The resting heart rate of 76/min as ncreased to 93/min during the 2 ½ m nutes period he was able to pedal a load of 300 kpm/min (fig 2) after wh ch he lost consc ousness and fell from the b cycle He recovered rap dly on resting sup ne and the test was repeated w th a similar sequence of events Later on exercise testing was per formed n the supine pos tion together with measurements of pressure flow relat onships (table I) The card ac output was measured by the F ck pr nc ple exp red air being collected into Douglas bags The patient tolerated the f rst load of 200 kpm/m n for five m nutes at the end of wh ch a fit of cramps occurred and the resp rat on became d scont nuous A per od of 10 m nutes was allowed before the second load of 400 kpm/min wh ch the pat ent tolerated for 3 minutes w th s milar symptoms Exp red air was collected betw een 3—5 minutes dur ing the first load and between 1—3 minutes during the second load No s gns of dyspnea were evident during the exerc se per ods



Fig 1 An aortography showing the delayed fill ng of the left vertebral ar ery rising f om the occlus on n the left subcla ian artery marked by the arrow

As is ev dent from the table I the pat ent was unable to ncrease the heart rate o er a limit of 91/min Further the resp ratory m n ute volume was not increased by the second load over that achieved already at the lo er

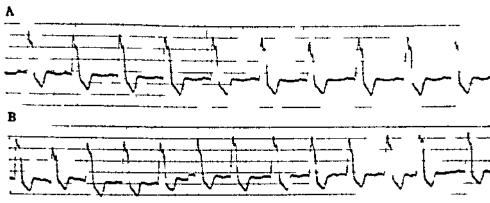


Fig 2 A he heart ra e in s nus rhythm of 76 m n. when the pa ent was s ng on the ergometer B the heart rate n s nus rhythm of 93 m n when the pa ent was pedall ng a load of 300 kpm/m n

TABLE I Respiratory and circulatory data at rest and during two successive exercise periods

	Rest legs on the pedals	200 (kpm/min)	400 (kpm/min)
Respiratory minute volume l/min	7 44	31 7	30 9
Oxygen uptake ml/min	230	761	711
Oxygen content vol %			
pulmonary arterial blood	15 0	10 1	10 8
brachial arterial blood	20 1	20 6	20 6
Cardiac output l/min	4 51	7 25	7 26
Heart rate beats/min	74	91	91
Stroke volume ml	60 9	79 7	79 8
Brachial arterial pressure, mm Hg	156/85 119	159/85 122	156/93 115
Total peripheral resistance dyn sec cm^{-5}	2 109	1 346	1,267

exercise level, and the cardiac output was essentially the same during the loads in spite of a 100 % difference in the mechanical work The reaction of the peripheral vascular bed was commensurate with the change in the total blood flow, and no blood pressure drop occurred

An intravenous injection of atropine in a dose of 1 0 mg raised the heart rate from 65/min to 90 min in one minute with no further rise during the following 5 minutes [...]utaneous epinephrine in a dose of [...]5 microg raised the heart rate by 12 beats/min

Discussion

In considering this case there hardly is any doubt that generalized atherosclerosis including the coronary arteries was the primary disease The convulsive response to muscular exertion evidently originated via cerebral hypoxia due to handicapped vertebral circulation This response is however, not the essence of the findings which demonstrated that cerebral pathology can distinctly modify the cardiopulmonary response to exercise

The respiratory regulation was exactly the clinical image of the findings in the hypothalamically injured dogs of Smith (16) Since the maximal breathing capacity obtained by voluntary ventilation was normal, the innervation of the ventilatory muscles must have been intact The fact that no further increase occurred in the ventilatory volume on increasing the mechanical work, even in the face of the short duration of the exercise speaks for a disarranged function of the higher integrating centers This is also evidence in favor of a central origin for the hyperpnea of exercise in general and especially at low levels of stress when no abnormalities exist in the blood chemistry (10)

The attempt to block the vagal activity did not accelerate the heart more than muscular exercise nor was the heart rate at rest low and concordant with vagal preponderance A centrally originated infrequent discharge through the sympathetic nervous system seems to be the most relevant explanation

The blockage of the terminal sympathetic neurones by guanethidine does not result in the hemodynamic picture present in this patient (6, 18) because accompanying peripheral effects interfere On the other hand complete cardiac denervation results in a limited heart rate response to exercise with enhanced stroke volumes (2) The stroke volume response to exercise in the patient was of the magnitude observed at exercise levels entailing a more than 6 fold increase in oxygen uptake (5), and was thus out of proportion to the external load The absence of a further rise in the stroke volume might be due to intrinsic myocardial disease evoked by coronary atherosclerosis but in the light of the findings of Rosen (12) and Manning et al (8) a centrally induced decrement in the myocardial contractile force may also be implicated

In the lack of metabolic data no explanation can be offered as to how the patient managed the three minutes of the heavier exercise with an unaltered cardiac output and oxygen consumption Already the first A V oxygen difference was abnormally large in proportion to the oxygen uptake and near the maximum found in normal subjects during supine leg exercise (3 5) Since no further widening of the A-V oxygen difference occurred during the second load an oxygen debt must have been developed despite the curious absence of dyspnea While the heart rate cardiac output and respiratory response were not gradable, the neurological signs appeared faster during the second load reflecting an earlier appearance of cerebral hypoxia

The heart rate has been shown to be fixed in exercise in patients with tabes dorsalis (13), and in idiopathic postural hypotension as can be judged from the pressure tracings of Marshall et al (9) Both these disease states are characterised by postural blood pressure reactions The present case and the one previously reported (4) show that the heart in *sinus rhythm* may respond with poor acceleration to muscular exercise in patients with intact peripheral vasomotion The evidence that this mismanagement can issue from supraspinal central nervous-system lesions of both inflammatory and ischemic origin advocates an objective exercise testing of patients with a history of exercise intolerance combined with central neurological signs

Summary

An exceptional case is reported with the following hemodynamic and respiratory findings the heart rate in *sinus rhythm* could not be raised over about 90 beats/min by muscular exercise, intravenous atropine or subcutaneous epinephrine the maximal minute ventilation increased only to 30 l/min in physical exercise the A V oxygen difference was abnormally large during light exercise and no graded increase occurred in heart rate cardiac output and oxygen uptake during heavier exercise A fit of cramps brief periods of unconsciousness and irregular breathing occurred during muscular exercise No postural blood pressure decline was demonstrable and the peripheral vascular bed reacted normally on effort

The patient had a facial paresis of central origin, and aortography revealed an inadequate vertebral circulation due to occlusion in the left subclavian artery In the light of the evidence gained from animal experimentation, the most probable mechanism evoking the abnormal exercise circulation and respiration was a disintegrating function of the supraspinal centers

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From the Department of Anaesthesiology (Head W H Dam) and the Department of Medicine B (Head M Bjorneboe), Bispebjerg Hospital Copenhagen and the University Institute of Pathological Anatomy (Head T Steen Olsen) Kommunehospitalet Århus Denmark

Liver Function, Histology, and Cytochemistry in Man Following Halothane and Cyclopropane Anaesthesia

By

M Stig Christensen, V F Askrog, T Steen Olsen, E Kemp and N Tygstrup

Halothane (Fluothane®, halothanum (NFN)) has been closely examined for hepatotoxicity because of its chemical structure (1,1,1,-trifluoro-2,2 bromo-chlorethane) Extensive animal experiments and the clinical experience from a vast number of anaesthesias with the drug have refuted the gravity of this risk (5 16, 18) In some cases however an association between halothane anaesthesia and liver failure has been demonstrated and the possibility of a toxic effect is widely discussed (15 17) Since the complication is very rare, the question is difficult to answer It has been suggested that the liver of some patients is hypersensitive to halothane in order to explain that jaundice in most cases has appeared following several halothane anaesthesias (7, 14) A slightly different explanation is that halothane is a weak hepatotoxin, and clinically manifest liver dysfunction therefore only develops under special conditions From the latter point of view a thorough search for slight, non fatal hepatic disturbances following halothane may be of interest

The aim of the present work was to study this question by means of liver function tests and histological and cytochemical examination of liver biopsies before, during and some days after halothane anaesthesia A control series in which cyclopropane was the anaesthetic agent, was studied similarly

Material

The material consists of 13 halothane anaesthesias and 14 cyclopropane anaesthesias The patients studied were selected by the following criteria 1) Good general condition with no manifest hepatic disease 2) upper laparotomies were excluded 3) the same anaesthetic had been given previously more than 2 weeks earlier

The age and sex of the patients the type of operation and the duration of the anaesthesia in the halothane and the cyclo-

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propane material appear from table I One patient (No 2) had a slight insulin treated diabetes of 18 years duration This may account for the histological changes in the liver biopsy specimen. Patients No 1 and No 22 had been cholecystectomized previously but had no symptoms of bile duct disease at the time of the study In patient No 25 a liver biopsy taken 3 years previously had shown hepatitis, possibly incipient cirrhosis. The actual liver tests and biopsy specimens were normal In patient No 17 the liver biopsies unexpectedly showed cirrhosis. The liver tests were normal, and there were no clinical signs of cirrhosis. The patients were questioned about their alcohol intake Two of them (No 11 and No 15) took several bottles of beer daily

Blood samples for liver tests and liver biopsy specimens were obtained in the morning before the anaesthesia was started during the operation immediately before administration of the anaesthetic was discontinued, and on the 8th postoperative day In two patients (No 4 and No 8) a fourth series was taken, 2 and 4 months after the operation, respectively

The postoperative course was uneventful in all patients except in No 4 and No 8 who were subfebrile (max. 38 C) one and two days after the operation. No ill effects ascribable to the investigational procedures were noted

Methods

Anaesthesia All patients were premedicated by Nembutal sodium® (mebumalnatrium (NFN) orally two hours before anaesthesia, 30 to 150 mg according to age and weight. Immediately before the anaesthesia was induced, they received atropine sulphate 1 mg i v and they had oral endotracheal intubation after relaxation with 20 to 40 mg of Tubarine® (tubocurarini chloridum (NFN

In the halothane group anaesthesia was induced by 2 per cent (v v) halothane from a Fluotec MK II vapourizer in nitrous oxide and oxygen (2 1) and it was maintained by 1 per cent (v/v) halothane Cyclopropane anaesthesia was induced by 40 per cent (v/v) cyclopropane in oxygen and maintained by 20 per cent (v/v) cyclopropane

The anaesthesia was administered in a semi-closed to-and fro-system with Water s absorber The respiration was controlled by a Bennett respirator with intermittent positive/negative pressures The tidal volume and respiratory rate were determined on the basis of a Radford nomogram, adding 10 per cent to obtain slight hyperventilation.

Arterial pCO (see table I) was measured by Astrup s method (2) and the oxygen saturation was determined spectrophotometrically (21) in samples withdrawn shortly before the peroperative liver studies

Biochemical liver tests Serum bilirubin (20), serum glutamic oxalacetic transaminase (12) and serum lactic dehydrogenase (11) were determined by the methods indicated The normal range for these methods are as follows serum bilirubin < 1 0 mg per 100 ml serum glutamic oxalacetic transaminase < 17 units and serum lactic dehydrogenase < 21 units

Histological examination Liver biopsies were taken by the Menghini technique The syringe contained a few ml of ice-cold 0 25 M sucrose The biopsy specimen was immediately divided and one half was fixed in 4 per cent aquous formaldehyde buffered with phosphate to pH 7 The tissue was dehydrated, embedded in paraffin and cut into 7—10 micron sections Routine staining with hematoxylin-eosin and van Gieson Hansen Most of the specimens were of good quality the length of the biopsy cylinder being at least 5 mm and generally much more (15—35 mm) The biopsies smaller than 5 mm are indicated in table III as small biopsy

The biopsies were processed and described together with routine biopsies from several medical departments. The examinations were performed by one pathologist (T.S O) who did not know the clinical data of the patient. After conclusion of the study all

specimens were re studied systematically but in no case did this change the original classification

Cytochemical examination The other half of the liver biopsy was immediately placed in ice-cold 0 25 M sucrose and homogenized in 2 ml of this medium a few minutes later by a micro-Potter Elvehjelm homogenizer The steel pestle was driven by a motor with 1 400 rpm while the container (immersed in ice) was moved slowly up and down This procedure performed for one minute produces a rather gentle homogenization

The homogenate was transferred to pre chilled plastic centrifuge tubes The homogenate was centrifuged at 900 × g for 10 min in a refrigerated centrifuge (International model PR 1) equipped with the multispeed attachment After the first centrifugation the pellet (unbroken cells nuclei erythrocytes cell debris) was discarded The supernatant was removed by a chilled Pasteur pipette to other pre-chilled plastic tubes kept in cracked ice and then recentrifuged at 30 000 × g for 10 min in order to separate mitochondria from cytoplasm The layer of fat which gathered on top of the supernatant was carefully removed The supernatant i e the cytoplasmatic' fraction which consists of cytoplasm light mitochondria ribosomes etc was diluted with 2 ml 0 25 M sucrose and stabilized with 100 μl of a 0 5 % solution of pure albumin The sediment i e the mitochondrial fraction was rehomogenized in 2 ml 0 25 M ice-cold sucrose in a glass homogenizer (1 400 rpm) for one minute The homogenate was stabilized by adding 100 μl of a 0 5 % solution of pure albumin Before stabilization of the two fractions samples of 100 μl were withdrawn for protein determinations by a modification of Lowry s method (8) The temperature was rigorously maintained at 0—1 centigrade The procedure represents a slight modification especially concerning the force of the centrifugations of the method described by Wembach (19)

In the cytoplasmic fraction the following enzymes were determined by the methods indicated Glutamic-oxalacetic transaminase (GOT) (12) lactic dehydrogenase LDH (11) alcohol dehydrogenase (ADH 1 In the mitochondrial fraction GOT was determined by the same method The activities are expressed in units per milligram of protein

Results

The halothane and the cyclopropane material are not entirely comparable (table I), since the female/male ratio is smaller in the halothane material, and likewise the mean age of the patients and the mean duration of the anaesthesia is smaller The larger fall in blood pressure in the halothane group is a well known pharmacological effect of this anaesthetic Hyperventilation is slightly more pronounced in the halothane group Hypoventilation as manifested by abnormally high pCO or low oxygen saturation in arterial blood, was not seen in any of the patients

The mean values of the liver tests examined (table II) are not significantly different in the halothane and the cyclo propane material and similarly the mean differences between samples Nos 1 2 and 3 are not statistically significant The mean postoperative serum bilirubin concentrations are slightly lower than the pre and peroperative concentrations This is mainly due to serum bilirubin levels slightly above the normal limit in the pre and peroperative samples from patients Nos 2, 4 9 14 20, and 27 No explanation of this finding can be given

Slightly abnormal levels of serum glutamic-oxalacetic transaminase were

TABLE I Patients studied

Patient no	Age (yrs)	Sex	Surgery	Duration of anaesthesia (min)	Max fall in B P (%)	pCO_2 (mm Hg)	*Oxygen saturation* (%)
Halothane							
1	32	♀	Genital prolapse	60	[1]38	36	93
2	61	♂	Revision of wound	40	[1]44	35	95
3	62	♂	Hemorrhoids	35	15	33	96
4	18	♂	Osteosynthesis	120	22	30	94
5	58	♂	Osteosynthesis	100	43	39	93
6	67	♂	Bladder stones	75	[1]29	29	95
7	37	♂	Dislocated humerus	130	12	27	94
8	55	♀	Osteosynthesis	85	28	33	95
9	18	♂	Pilonidal cyst	60	13	31	99
10	51	♂	Osteosynthesis	125	45	33	95
11	50	♂	Anal fistula	60	36	27	95
12	19	♂	Pilonidal cyst	45	27	32	99
13	58	♀	Osteosynthesis	70	35	35	94
Mean	45			77		32	95
Cyclopropane							
14	47	♂	Ventral hernia	90	0	34	97
15	41	♂	Ventral hernia	140	0	36	97
16	50	♀	Mastectomy	100	10	37	97
17	57	♀	Genital prolapse	65	0	43	96
18	36	♀	Ventral hernia	80	0	38	95
19	29	♀	Hysterectomy	65	0	36	96
20	53	♂	Inguinal hernia	95	0	38	97
21	44	♂	Inguinal hernia	95	0	39	98
22	67	♀	Stripping of varices	105	0	37	97
23	74	♀	Genital prolapse	85	0	36	97
24	71	♀	Mastectomy	90	5	41	96
25	72	♂	Inguinal hernia	85	0	35	97
26	76	♂	Inguinal hernia	100	0	35	96
27	63	♂	Inguinal hernia	85	20	34	96
Mean	56			91		37	97

[1] Vasopressors have been given

found in 4 patients (Nos 5, 9 24, and 26) In patient No 5 there is a great difference between the pre and peroperative determination, and one of them may be erroneous In patients Nos 24 and 26 mild histological changes were

TABLE II Liver tests before during and after anaesthesia

Test	Bilirubin (mg/100 ml)				S GOT (units)				S LDH (units)			
Sample no	1	2	3	4	1	2	3	4	1	2	3	4
Patient no	Halothane											
1	0 5	0 6	0 6		0 8	1 3	0 8		12	16	16	
2	1 3	1 2	1 1		1 3	1 2	0 4		20	17	15	
3	0 9	0 9	0 3		1 0	1 1	1 3		15	—	12	
4	1 4	1 5	1 1	0 7	0 8	1 0	1 0	1 1	8	8	9	8
5	0 5	0 5	0 6		2 1	0 5	1 7		20	15	19	
6	0 5	0 6	0 5		1 2	1 1	1 5		11	14	15	
7	1 0	1 0	0 5		0 9	1 0	1 7		15	15	14	
8	0 4	0 4	0 4	0 5	0 8	1 0	7 0	1 1	14	13	27	15
9	1 6	1 7	1 0		—	0 6	2 4		10	10	12	
10	0 4	0 4	0 5		1 0	1 0	1 9		11	14	15	
11	0 7	0 7	0 7		1 1	0 8	1 4		11	9	14	
12	0 9	1 0	0 6		1 3	1 2	3 7		10	14	14	
13	0 5	0 5	0 4		0 6	0 6	0 9		20	15	16	
Mean	0 8	0 9	0 6		1 1	1 0	2 0		14	13	15	
SEM	0 11	0 11	0 07		0 11	0 06	0 48		1 1	0 8	1 2	
	Cyclopropane											
14	1 2	1 4	0 9		1 8	1 4	2 5		17	15	20	
15	1 0	1 2	0 6		0 8	1 2	1 0		14	13	11	
16	0 7	0 6	0 6		1 0	1 1	1 0		13	12	13	
17	0 8	0 7	0 5		1 6	0 9	1 1		10	13	9	
18	0 4	1 0	0 4		1 0	0 8	1 1		11	13	13	
19	0 4	0 3	0 4		—	1 9	0 5		13	15	11	
20	2 0	2 2	0 5		1 3	1 2	1 6		13	12	11	
21	0 7	0 7	0 7		1 4	0 8	1 9		15	10	14	
22	0 8	0 9	0 7		1 0	0 8	0 7		13	14	18	
23	0 4	0 4	0 3		0 8	0 8	1 2		19	21	16	
24	0 7	0 6	0 9		2 9	2 9	1 2		14	11	20	
25	0 7	0 8	0 6		1 7	0 5	0 8		11	11	11	
26	0 5	0 6	0 8		1 8	2 2	1 2		20	17	20	
27	1 1	1 1	1 0		1 1	1 3	1 9		20	14	13	
Mean	0 8	0 9	0 6		1 4	1 3	1 3		15	14	14	
SEM	0 11	0 14	0 05		0 16	0 18	0 11		0 9	0 8	1 0	

found but they did not exceed the changes found in some other cases which had normal S GOT values Among the postoperative determinations abnormal values were found in 4 patients of the halothane group and 3 of the cyclo propane group (Nos 8 9 10 12 14, 21 and 27) The elevation was marked in patient No 8, moderate in No 12 and borderline in rest of the cases

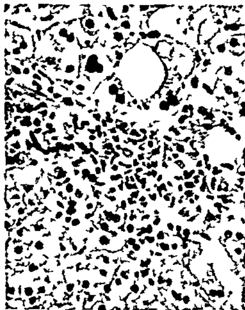
TABLE III Liver histology before during and after anaesthesia

Pat no \ Sample no	1	2	3	4
	Halothane			
1	Normal	Normal	Normal	
2	Moderate congestion Slight fatty change	Moderate congestion Slight fatty change	Moderate congestion Slight fatty change	
3	Normal	Normal	Normal	
4	Normal	Normal	Moderate fatty change	Normal
5	Normal	Normal	Normal	
6	Slight fatty change	Slight fatty change	Slight fatty change	
7	Slight fatty change	Slight fatty change	Slight fatty change	
8	Slight fatty change	Slight fatty change	Slight fatty change Multiple focal necroses	Slight fatty change (no necroses)
9	Normal	Normal	Normal	
10	Moderate brown pig mentation	Moderate brown pig mentation	Moderate brown pig mentation	
11	Normal	Normal	Normal	
12	Normal	Normal	Normal	
13	Normal	Normal	Normal	
	Cyclopropane			
14	Severe fatty change	Severe fatty change	Severe fatty change	
15	Normal	Normal	Normal	
16	Normal	Normal	Normal	
17	Normal (small biopsy)	Normal (small biopsy)	Possible cirrhosis (small biopsy)	
18	Normal	Normal	Normal	
19	Normal	Normal	Normal	
20	Normal	Normal	Normal	
21	Slight fatty change	Slight fatty change	Slight fatty change	
22	Normal	Normal	Normal	
23	Normal	Normal	Normal	
24	Slight fatty change	Slight fatty change	Slight fatty change	
25	Normal	Normal	Normal	
26	Moderate periportal lymphocytic infiltr	Moderate periportal lymphocytic infiltr	Moderate periportal lymphocytic infiltr	
27	Moderate fatty change	Moderate fatty change	Moderate fatty change (small biopsy)	

Among the determinations of serum lactic dehydrogenase only one, viz the postoperative sample in patient No 8, showed a significant increase



F g 1 Pa en No 4 th d b opsy Modera e fa y change Hema oxyl n-eos n s a n ng



F g 2 Patien No 8 th d b opsy Focus w h l ver cell damage and necrosis followed by local leucocyte nfil ra on and p ol fera on of Kupffer cells Hema oxyl n-eos n s a n ng

The histological find ngs are shown in table III S gn ficant changes in con secut ve b opsies were found only in two pat ents Pat ent No 4 showed mod erate fatty changes n the postoperative spec men (fig 1) and in pat ent No 8 there were mult ple focal necroses post operatively (fig 2) Control b ops es a couple of months later showed that these changes were revers ble

The other abnormal t es found proved to be very constant In 8 patients pre operative b ops es showed fatty in filtration i e t p cal vacuol zat on as shown n f g 1 Sta n ng for fat was not done since paraff n was used for em bedding of the biops es In one patient (No 2) the d abetes may be the cause of the fatty change The moderate congest on found had no cl n cal cor relate In the other pat ents the fatty in filtration wh ch was pronounced in 2 pat ents (No 14 and No 27 could not be explained from the cl n cal informa tion Both pat ents who adm tted to be beer-drinkers No 11 and No 15) showed no abnormal ties

Bro vn pigmentation was not ced in several b ops es but apart from No 10 the p gmentat on was so sl ght that t can safely be regarded as normal

In patient No 17 the find ng of altera tions suggesting c rrhosis were unexpec ted As in the original descr pt on the d agnos s is only mentioned in the post operative b opsy which was the largest one The first t o b ops es do not con trad ct this d agnosis but are too small to contribute to it Pat ent No 25 in whom an incip ent cirrhosis was suspec

TABLE IV Liver cell enzymes before during and after anaesthesia

Enzyme (spec act)	GO transaminase (Mitochondria)				GO transaminase (Cytoplasm)			
Sample no	1	2	3	4	1	2	3	4
Patient no	Halothane							
1	19	22	28		52	52	41	
2	35	37	31		60	52	53	
3	32	25	52		52	61	59	
4	25	22	34	17	53	56	71	64
5	30	29	32		71	67	49	
6	26	46	22		63	51	66	
7	38	28	27		55	39	91	
8	36	28	27	50	61	59	61	70
9	43	49	29		—	68	66	
10	30	21	29		72	66	59	
11	30	29	33		54	68	44	
12	44	36	32		70	53	74	
13	22	23	32		56	64	61	
Mean	32	30	31		60	58	61	
SEM	2 1	2 5	1 9		2 1	2 4	3 7	
	Cyclopropane							
14	29	24	22		45	42	45	
15	72	40	24		93	73	62	
16	35	33	14		64	60	58	
17	10	24	17		54	58	51	
18	30	17	18		35	26	45	
19	58	90	50		73	87	61	
20	38	33	27		48	58	58	
21	44	38	30		74	44	59	
22	40	31	25		67	55	67	
23	7	15	24		57	45	63	
24	29	37	25		42	44	39	
25	33	32	21		33	53	119	
26	40	—	66		52	—	79	
27	44	38	36		49	56	65	
Mean	36	35	29		56	54	62	
SEM	4 4	5 1	3 7		4 4	4 2	5 2	

ted from a liver biopsy taken 3 years previously, now showed completely normal histological picture in 3 biopsy specimens of satisfactory size

The periportal infiltration with lymphocytes in patient No 26 was not associated with known hepato biliary disease It is unknown whether it may be ascribed to his old age (aged 76 years he is the oldest patient of the material)

The cyto chemical determinations appear from table IV There is no signif

Lactic dehydrogenase (Cytoplasm)				Alcohol dehydrogenase (Cytoplasm)			
1	2	3	4	1	2	3	4
40	35	74		465	435	441	
84	83	53		396	378	566	
18	81	16		317	870	728	
78	77	41	78	641	763	597	771
78	87	68		138	601	427	
111	234	111		557	697	487	
62	59	91		506	302	759	
153	91	112	100	546	439	281	434
—	47	62		—	463	1 364	
86	91	44		827	1 045	790	
37	40	34		708	560	671	
63	64	24		707	632	538	
142	110	176		996	1 036	1 051	
79	85	70		567	632	669	
11	14	12		64	66	79	
9	8	11		772	462	354	
41	29	46		563	545	608	
108	108	111		950	829	1 049	
106	92	89		1 076	738	911	
21	29	87		845	396	485	
43	31	71		415	371	642	
78	81	44		573	1,201	721	
90	78	70		503	380	741	
122	85	103		891	651	814	
16	5	202		49	22	925	
52	74	43		352	262	541	
68	64	61		165	1 047	2 042	
48	—	34		280	—	217	
68	58	65		384	404	114	
62	57	74		558	562	726	
10	9	12		80	90	124	

icant difference between the values in the halothane and the cyclopropane materials nor are the differences between the mean values of the consecutive biopsy samples statistically significant in any case When the individual changes in activity are calculated the mean fall in mitochondrial GOT activity from sample No 2 to sample No 3 appears to be significant in the cyclopropane material in all other cases the changes are not statistically significant

Discussion

In the present material no case of unequivocal liver failure was observed, nor could this be anticipated, since this complication is very rare The question is, if our observations indicate even minor hepatic involvement in relation to anaesthesia and operation In the majority of the patients no evidence of hepatic involvement was demonstrated, but in a few patients some alterations in the parameters studied were observed

The interpretation of observations of this nature can only be tentative Normal results do not exclude that other tests, or the same tests performed at other intervals, might have revealed liver damage Likewise abnormal tests do not necessarily indicate liver damage, as they may be due to extrahepatic factors If abnormal results do reflect liver injury, they still fail to reveal the cause of the injury In this and similar studies the patients are under the influence of many factors other than anaesthesia, such as their primary disease, preoperative investigations premedication, surgery, blood transfusion etc

Is it likely that the liver tests used are able to reveal slight liver injury? Serum bilirubin is a rather insensitive test of injury to the parenchymal liver cells, and the negative findings therefore do not exclude such injury Serum transaminases usually rise in response to relatively slight tissue injury but this is not specific of liver injury, and the rise may be short lasting During the first postoperative week, i e the interval between the second and the third sample, minor elevations may have disappeared This interval was chosen in order to avoid unspecific reactions caused by surgery (13), and because 'halothane hepatitis' in most cases has become manifest at that time The serum lactic dehydrogenase is less sensitive to liver injury than are transaminases, and it was mainly performed in order to compare it with the cytochemical determinations Great importance therefore cannot be attached to slight and isolated changes in the liver tests, and only the findings in patient No 8 can with some confidence be assumed to reflect liver damage It is of interest to note that this patient also exhibited the most conspicuous changes in the liver biopsy

The light microscopical picture of the liver cells is probably not a very sensitive measure of transient liver damage, but the focal necroses found in patient No 8 are conclusive The fatty change found in the postoperative specimen in patient No 4 also may be a result of liver damage, but in this patient it is not supported by abnormalities of the liver tests On the other hand, the postoperative biopsy specimen of patient No 12 who had a moderate increase in serum transaminase, is unchanged We consider patients No 4 and No 12 as cases of possible liver damage

The cytochemical studies have been of little help in this connection Changes in liver enzyme activities following liver damage have been demonstrated (3, 6, 9) and it is reasonable to expect that cytochemical determinations will prove to be the most specific and sensitive tests for liver injury of any kind, but great theoretical and practical difficulties must first be solved No significant changes in the cell enzymes were found

and the scatter of the values is considerable In the latter respect our data agree well with the findings of other authors It should be noted that the enzyme pattern in patient No 8, who has probable liver damage, and in patients Nos 4 and 12 in whom liver damage is thought possible, is unremarkable

Is halothane the cause of the supposed liver damage? Under the circumstances of the present study the question only can be answered by statistical comparison of the halothane and the cyclopropane group With only one probable and two possible cases of liver damage in the halothane group it is obvious that the groups are much too small for statistical analysis It is suggestive, however, that similar cases were not seen in the cyclopropane group, despite the older age of these patients, the longer duration of the anaesthesia and the inclusion of one patient (No 17) with pre existing liver disease and one (No 25) with previous hepatitis verified on biopsy

If one accepts that the patients mentioned suffered from halothane induced liver damage, it still may be questioned whether this represents an abortive form of 'halothane hepatitis The histological appearances of the first postoperative liver biopsy specimen in patients No 4 and No 8 viz fatty change and focal necroses are not typical for the alterations described as halothane hepatitis which is mostly described as a diffuse lesion (4) but fatty change and focal necroses (10) have also been noted Only the two patients mentioned had a slight postoperative febrile reaction, for which no surgical explanation was demonstrated Postoperative fever is a typical feature of 'halothane hepatitis' (14), but of course it is of small diagnostic significance in this context The fact that the histological and biochemical lesion was fully reversible does not exclude that halothane was the cause

Conclusion

The study demonstrates that special investigations not rarely may reveal postoperative liver damage in some patients in whom this was not suspected from the usual clinical observations There is some evidence to support the conception that this liver damage is halothane induced but a statistical proof apparently will require so large materials that the present plan of study, which includes repeated liver biopsies is impracticable

Slight halothane induced liver damage thus may be much more common than 'halothane hepatitis but so far this has not helped to elucidate the mechanism of the lesion, since it cannot be explained why some patients are susceptive and others are not Careful revision of the preoperative clinical data of afflicted patients gave no clue to this question In the patients with possible pre existing liver dysfunction i e patient No 2 (diabetes) and patient No 11 (constant intake of alcohol), the investigation was entirely negative

Summary

The effect of halothane anaesthesia on the liver was studied in 13 patients without manifest liver disease Fourteen

patients receiving cyclopropane anaesthesia for surgical operations of the same type as in the halothane group (i e of moderate duration and not involving splanchnic or thoracic viscera) served as controls In all patients the anaesthetic in question had been given on a previous occasion Liver tests and liver biopsies were taken before, during and one week after the anaesthesia

In one patient in the halothane group the serum GO transaminase showed a moderate increase, and the liver biopsy showed focal necroses On reexamination 2 months later these signs of liver injury had disappeared

One patient in the halothane group showed fatty change in the postoperative specimen, not seen in two previous biopsies and one taken 3 months later The liver tests remained within normal limits

One patient in the halothane group had a slight increase in the postoperative serum GO transaminase value, the liver biopsies showed no changes

The liver cell enzymes showed no consistent change in any of the patients

In the cyclopropane group no significant changes in the liver test and histology were demonstrated

The relationship of these findings to 'halothane hepatitis is discussed

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From the Coagulation Laboratory (Head Inga Marie Nilsson M D) of the Department of Medicine (Head Jan Waldenstrom M D) University of Lund Allmanna Sjukhuset Malmo Sweden

Studies on the Platelet Adhesiveness in von Willebrand's Disease

By

Stig Cronberg, Inga Marie Nilsson and Jorgen Silwer

In von Willebrand s disease, an auto somal dominant hereditary bleeding dis order, the bleeding time is prolonged and the AHF (factor VIII) is decreased (12 13, 14 15 16, 17)

As first shown by Nilsson and co workers (12, 14 15) in Sweden it is possible to correct not only the AHF deficiency, but also the prolonged bleed ing time and capillary bleeding by in jecting AHF containing human fraction 1—0 prepared by the glycine meth od of Blomback and Blomback (1) In later studies they found that the prolonged bleeding time in von Wille brand s disease is due to the lack of a plasma factor present in normal plasma as well as in haemophilia A plasma and that this factor is not identical with AHF, fibrinogen or platelets These find ings have since been confirmed by others (4 5)

The mode of action of the plasma factor lacking in von Willebrand s dis ease is not known Nilsson and co workers (11) thought that it might act on the capillary wall or on the platelets or on both To demonstrate the factor it was therefore necessary to resort to the use of transfusions in patients with von Willebrand s disease and in many laboratories extensive endeavours have been made to find a reliable method for measuring the factor in vitro Such a method would be useful in the diagno sis of the disease and also for estimating the factor in various plasma prepara tions, and would probably help us to understand the many peculiarities of the disease and to explain the role of the factor in the mechanism of haemostasis

When measuring the bleeding time in patients with von Willebrand s disease Borchgrewink (2) noticed that the con centration of the platelets in shed blood was as high at the beginning as towards the end of the bleeding This suggested the occurrence of some form of decreased adhesiveness of the platelets in vivo

Attempts have therefore been made to demonstrate a decreased platelet adhesiveness also in vitro These attempts

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have been based mainly on the adhesiveness of the platelets to glass Hellem (8) passed citrated blood through a plastic tube filled with glass beads and found the adhesiveness of the platelets to be slightly decreased in von Willebrand's disease Salzman (23) allowed blood to run directly from the sampling needle through such a filter with the aid of a vacutainer and found the adhesiveness of the platelets to be markedly decreased in his patients Zucker (27), who used the same method as Hellem, at first found a substantial decrease in the adhesiveness, but she could not reproduce her results A similar method was also used by O Brien (22), who found the adhesiveness of the platelets to be normal in all three patients studied

After Norwegian scientists (6) had shown that ADP aggregates platelets, Vainer and Caen (26) studied the optical density of platelet rich plasma after addition of ADP and found a decrease in optical density owing to aggregation of platelets At high concentrations of ADP the optical density decreased both in normals and in patients with von Willebrand s disease, but at a low concentration, i e at about a final concentration of 0 05 μg ADP/ml plasma, there was a slight but inexplicable increase in the optical density of plasma from patients with von Willebrand's disease, while there was a decrease in that of plasma from normals Meyer (10) in Paris, however, later found 4 patients out of 26 with von Willebrand's disease not to differ from normals in this respect

Ödegaard et al (28) added ADP to a corresponding final concentration, 0 05 μg/ml platelet rich plasma, and passed the plasma through a glass bead filter They then found that platelets from patients with von Willebrand's disease showed no adhesiveness at all, while that of platelets from normals was on the average 25 per cent Their original report was based on 5 patients When ADP was added to a higher final concentration they found platelets from patients with von Willebrand's disease to aggregate in a normal way

Since we in Sweden are particularly interested in this disease and since we have so far investigated and classified 200 cases of von Willebrand's disease in members of 88 Swedish families, we decided to extend the investigation to include the estimation of the platelet adhesiveness in this disease, mainly by Hellem s original whole blood method and by his ADP plasma method, in 68 cases of the disease

Material and methods

Human fraction I—0 Human fraction I—0 containing AHF, was prepared at the Chemistry Department II, Karolinska Institutet, Stockholm by the glycine method of Blomback and Blomback (1) One dose of fraction I—0 is prepared from 1 400 to 1 600 ml of fresh normal plasma and contains about 3 g of protein Usually one half or one dose of fraction I—0 was given on each occasion Half a dose of fraction I—0 dissolved in 100 ml of isotonic saline has an AHF activity 5 to 8 times that of 100 ml of fresh normal plasma

Collection of blood Blood was obtained from a vein after application of a tourniquet which was left in place for at most 2 minutes Venipuncture was performed with sharp wide siliconecoated needles (Chrom Acufirm No

1 8) No syringe was used The puncture was made with a firm quick stab in order to secure a rapid steady stream and not simply a trickle of blood The first few millilitres of blood obtained were discarded, after which the blood was allowed to flow directly into a series of siliconized 15 ml centrifuge tubes having a 10 ml mark and containing, unless otherwise stated 1 ml of 3 8 per cent trisodium citrate solution The blood was allowed to run to the 10-ml mark, and each tube was then immediately inverted twice against a square of plastic sheeting

Coagulation tests All the methods used for preparing the blood samples and for determining the various coagulation factors have been described elsewhere (18) The *AHF activity* of plasma was assessed by its normalizing effect on the recalcification time of haemophilia A plasma (15, 18 19), and the amount of AHF present was expressed as per cent of that found for a normal standard consisting of a pooled plasma from 10 normal individuals One method of determining the *bleeding time* was that of Duke using standardized haemolets (Dade Reagent Inc Miami Florida U S A) Determinations were performed on both ears Normal range 1 to 4 minutes Use was also made of the method of Ivy as modified by Borchgrevink and Waaler (3 20) Cuts were made with a surgical blade (Gilette Surgical Blade E) each blade being used for one examination only The bleeding times of 3 transversal standard cuts 1 mm deep and 10—14 mm long were measured and the mean of the three values noted was taken as the bleeding time of the patient The overall mean for such triplicate determinations in 35 normal individuals was found to be 9 5 minutes (range 5—15 5 minutes)

Platelet adhesiveness This was measured by a slight modification of the method of Hellem (8) According to this method citrated whole blood is passed through a standardized glass bead column at a constant rate Some platelets then adhere to the glass beads and the percentage of adhesive platelets is calculated from the platelet counts noted before and after the passage The glass beads for the filters were the same as those used by Hellem (Reflexperlen 31/7 Dragon Werk Bayreuth Germany) but they were not washed before use Each filter contained 4 g of glass beads Otherwise the filters were prepared in exactly the same way as those used by Hellem One modification consisted in the use of a slightly higher concentration of the sodium citrate (3 8 %) than Hellem (3 13 %) All our patients had haematocrit values within the normal range The citrated whole blood was allowed to stand at room temperature for an hour and was then sucked up into a siliconized syringe and passed through the filter with the aid of a machine to secure an absolutely constant speed The reason why we let the blood stand for one hour was that we found that the adhesiveness was fairly constant and optimal 30 minutes to 2 hours after the sampling The blood was collected from the filter for 26 seconds during which period 1 ml blood was passed through into tubes containing 19 ml of 3 8 % sodium citrate solution These tubes were allowed to stand at room temperature for 2—6 hours The platelets were then counted under the phase contrast microscope in accordance with Hellem s (8) modification of Nygaard s (21) method

Platelet adhesiveness in platelet rich plasma This was likewise performed by a method described by Hellem et al (9) In platelet rich citrated plasma there is no adhesiveness but when ADP is added in various amounts the platelets adhere to the glass and aggregate (9)

A stock solution of ADP (Sigma Chem Corp) containing 400 μg per ml was prepared in isotonic TRIS-buffer solution of pH 7 4 This solution was frozen in portions of 1 ml Each portion was thawed and diluted with the buffer solution to the desired concentration before use

The platelet rich plasma was prepared from citrated blood collected in the way described above and immediately centrifuged for 10 minutes at 185 *g* The plasma was

TABLE I Platelet adhesiveness and data on the individual cases of von Willebrand's disease

Fam no	Coordinate no	Published in	Sex	Initials	Year of birth
2 (B)	IV 3	15	♀	G L	50
3 (C)	IV 1	15	♀	M H K	38
5 (E)	IV 7	15	♂	P P	19
5 (E)	V 1	15	♂	W P	47
9 (I)	V 19	16	♀	E S	38
10 (J)	V 6	16	♀	M A	32
11 (N)	III 1	14	♂	G B	13
13 (M)	VI 20	14	♂	S E	35
14 (K)	II 4	14	♂	B H J	11
14 (K)	III 5	Nilsson et al (to be publ)	♂	B G J	41
14 (K)	III 6	Nilsson et al (to be publ)	♂	L J	57
15		Nilsson et al (to be publ)	♂	K S	34
16 (P)	IV 3	14	♀	A K Z	46
20 (T)	III 1	14	♂	G P	09
28	III 3	20	♂	A S	15
28	IV 15	Nilsson et al (to be publ)	♀	I N	39
28	IV 16	Nilsson et al (to be publ)	♀	A S	47
29		Nilsson et al (to be publ)	♀	I G K P	26
35	IV 5	20	♂	P T	05
42	II 4	24	♀	H N	99
42	II 5	24	♀	A S	03
42	II 7	24	♂	I A	06
42	II 8	24	♀	S P	08
42	III 2	24	♂	I N	13
42	III 4	24	♀	A F	23
42	III 6	24	♀	A G K	21
42	III 13	24	♀	I S	36
42	III 21	24	♂	S N	26
42	III 22	24	♀	M P	28
42	III 25	24	♀	S H	37
42	III 26	24	♀	H I H	40
42	III 27	24	♂	K Z	30
42	III 28	24	♀	K E	39
42	III 31	24	♀	I S	32
42	III 32	24	♂	N O A	34
42	III 33	24	♂	B A	37
42	III 34	24	♀	K S	40
42	III 36	24	♀	I B K	34
42	III 38	24	♂	J I A	46
42	III 39	24	♀	L A	48
42	III 41	24	♀	M P	53

Bleeding time (min)		AHF	Adhesiveness (%)			
			Plasma + ADP ADP (μg/ml)			Citrated whole blood
Duke	Ivy	(%)	0.2	0.1	0.05	
>30	>30	4	77	67	47	24
>30	>30	7	60	39	21	30
30	>30	21	55	26	11	32
4 >30	>30	15	76	46	22	45
>30	>30	2–9	76	53	29	23
>30	>30	15				35
>30	>30	10	72	54	22	15
>30	>30	8	79	53	27	19
6–14	>30	10–46	67	42	26	23
9	>30	17–32	44	17	8	15
7–20	>30	10–45	79	69	36	32
5–7	>30	50	75	62	32	45
>30	>30	4	80	56	38	22
3–16	20	39–46	74	69	44	45
30	>30	16–24	69	52	25	36
4–8	17	27	65	33	12	31
3–10	—	50	62	45	22	22
1–8	22	40	78	33	9	41
4 >30	>30	8–20	76	44	12	23
8	>30	43	68	42	19	23
2	>30	47	65	55	30	31
4	25	38	84	58	39	25
2	>30	30	77	68	40	44
3	30	48	36	18	3	42
5	>30	36	83	73	37	25
7	>30	30			23	28
3	28	45	71	42	14	36
5	25	49	69	41	6	19
4	25–>30	53	60	41	17	28
10–>30	>30	30		57	19	32
9	>30	43	66	42	10	28
3	>30	42	66	48	12	29
3	>30	29	70	47	24	26
4	24	44	70	60	44	25
5	>30	41	58	43	29	25
3	24–28	30–28	77	48	20	28
8	>30	35	79	54	45	29
4–7	>30	36	77	42	27	27
3	>30	44	59	39	30	31
3–15	17–>30	41	78	66	53	56
3	>30	55	77	65	46	33

Table I Cont

Fam no	Coordinate no	Published in	Sex	Initials	Year of birth
42	IV 7	24	♂	J F	49
42	IV 24	24	♀	B S	58
42	IV 25	24	♀	C S	59
42	IV 34	24	♂	J S N	47
42	IV 39	24	♂	M Z	59
42	IV 46	24	♂	M E	58
42	IV 50	24	♂	L S	56
42	IV 51	24	♂	M S	59
42	IV 59	24	♂	C A	57
42	IV 60	24	♂	A A	60
42	IV 61	24	♂	P S	58
45	III 6	Nilsson et al (to be publ)	♂	R O	47
49	IV 1	Nilsson et al (to be publ)	♀	A E	10
49	IV 3	Nilsson et al (to be publ)	♀	A O	17
56		Nilsson et al (to be publ)	♂	D H	02
57		Nilsson et al (to be publ)	♂	K Å A	19
58		Nilsson et al (to be publ)	♂	I F	51
58		Nilsson et al (to be publ)	♀	I F	54
58		Nilsson et al (to be publ)	♂	A F	57
59		Nilsson et al (to be publ)	♂	T A	44
60		Nilsson et al (to be publ)	♂	M J	56
66		Nilsson et al (to be publ)	♀	M S	15
71		Nilsson et al (to be publ)	♀	H J	45
78		Nilsson et al (to be publ)	♂	H A	57
82		Nilsson et al (to be publ)	♂	A T	55
83		Nilsson et al (to be publ)	♀	L L	4[illegible]
88		Nilsson et al (to be publ)	♀	I H	16

carefully withdrawn by means of siliconized drop-pipettes and transferred to siliconized tubes In order to obtain more reproducible values both ADP solutions and the tubes with the platelet rich plasma were kept at 4° for 30 minutes this cooling reduces ADP degradation when the solutions are mixed with the plasma 4 5 ml of the platelet rich plasma was then mixed with 0 5 ml of the ADP solution and immediately passed through the column Otherwise the performance of the test was the same as with the whole blood method ADP was a rule added to final concentrations of 0 2 0 1 and 0 05 μg per ml

Aggregation of platelets with collagen Human fascia was homogenized About 0 5 g of the tissue was suspended in 10 ml of 0 9 per cent saline The solution was then centrifuged at 2 000 *g* for 15 minutes The slightly opalescent supernatant was used

Bleeding time (min)		AHF	Adhesiveness (%)			
			Plasma + ADP ADP (μg/ml)			Citrated whole blood
Duke	Ivy	(%)	0 2	0 1	0 05	
2–8	25	48	76	41	25	29
3	–	38			34	31
2	–	56		37		23
3–25	25–>30	43		35	7	21
4–>30	>30	20–49				29
4	>15	29				25
6–8	23	50			37	35
3	28	49			53	40
3–5	20	60	81	39		29
2–9	>30	55			22	25
8–15	26	18	84	68	41	28
>30	>30	23				13
2–9	15	52–63	62	53	22	33
4–6	11	50	77	63	28	44
>30	>30	23				13
3–7	>30	13				27
6	>30	48				41
4–7	>30	55				12
7–30	>30	35				30
3–6	>30	44				26
>30	>30	35				20
2	22	59	79	58	31	42
3–6	18	49	71	52	27	18
3–4	>30	17	82	71	47	
3	22	37	84	51	28	30
3–8	18	52	72	49	22	26
3–5	20 >30	42	69	52	21	43

Clinical material

The clinical material consisted of 68 patients with clinical and laboratory findings indicating a diagnosis of von Willebrand's disease according to the definition of the disease given by Nilsson and Blomback (13) i e prolonged Ivy bleeding time AHF deficiency and an autosomal dominant hereditary pattern The family history symptoms and coagulation status of most of the patients have been described in earlier works (14 15 16 24) These patients are referred to by the same initials year of birth and family number as were given in the earlier papers A survey of all the Swedish cases of von Willebrand's disease is being prepared The patients not described in earlier papers are referred to by the family number given in that work Several of our patients had been treated with fraction I—0 or fresh plasma and had all responded with

TABLE II Platelet ahesiveness in von Willebrand s disease

	ADP (µg/ml)	Patients with von Willebrand s disease No of subjects	Mean (%)	S D	S E	Normal No of subjects	Mean (%)	S D	S E
Citrated whole blood	—	67	29 1	± 9 3	±1 1	58	30 0	± 3 6	±0 8
Plasma	0 2	49	71 2	± 9 9	±1 4	47	69 2	±10 5	±1 5
+	0 1	52	49 6	±13 0	±1 8	63	48 1	±14 8	±2 1
ADP	0 05	55	26 9	±13 1	±1 8	63	25 9	±12 3	±1 6

TABLE III Platelet adhesiveness in patients with von Willebrand s disease before and after treatment with fraction I—0

	Before Adhesiveness (%)				After fraction I—0 Adhesiveness (%)			
		Platetet rich plasma +ADP (µg ADP/ml)				Platetet rich plasma +ADP (µg ADP/ml)		
Case	Citrated whole blood	0 2	0 1	0 05	Citrated whole blood	0 2	0 1	0 05
G B fam 11 (mean of 3 experiments)	14	74	55	22	20	75	53	17
I N fam 28	35	28	18	16	30	22	13	13
I G K P fam 29	32				40			

normalization of the prolonged bleeding time and increase of the AHF

The patients have been classified as severe or mild according to the prolongation of the Duke bleeding time (13) as follows

severe von Willebrand s disease the Duke bleeding time prolonged to more than 20 minutes,

mild von Willebrand s disease a slight or moderate prolongation of the Duke bleeding time up to 20 minutes

The material of normals (63 persons) consisted of medical students military orderlies laboratory technicians and members of the staff Most of them were 20—30 years of age

Results

The results are shown in tables I—IV Neither with the plasma ADP method nor with citrated whole blood was any difference found between the 68 patients with von Willebrand's disease and the normals Nor did we find any significant differences between the mild and severe cases (table I) Fraction I—0 given on 5 occasions to three patients normalized the otherwise prolonged bleeding time, but had no consistent effect on the adhesiveness of the platelets (table III)

Nor did addition of fraction 1—0 to plasma from normal controls increase the adhesiveness of the platelets in vitro (table IV)

Since our results would not tally with those reported by the Norwegian investigators, 5 of our severe cases in Sweden (i e with Duke bleeding time more than 30 minutes AHF between 3 and 20 % and with a typical hereditary pattern and severe bleeding symptoms) were examined in Oslo together with normals jointly by us and by our Norwegian colleagues, Dr Ödegaard and Dr Hellem under supervision of Professor Stormorken and Professor Owren One patient and one control were examined twice The examination was coded Neither our Norwegian friends nor we could make the diagnosis von Willebrand's disease in the patients in this mixed material In other words their earlier results could not be reproduced in these Swedish patients with severe von Willebrand's disease

TABLE IV Platelet adhesiveness in normal platelet rich plasma before and after addition of fraction 1—0

Mean values of 7 experiments

	Adhesiveness (%) Platelet rich plasma +ADP (µg ADP/ml)	
	0 1	0 05
Before	45	25
After addition of fraction 1—0	41	19

To 15 ml portions of fresh normal platelet rich citrated plasma was added 3 ml of dissolved fraction 1—0 (half a dose was dissolved in 100 ml of isotonic saline) and 3 ml of saline (the control sample before) respectively

Discussion

The results of investigations of platelet adhesiveness in von Willebrand's disease differ from one laboratory to another As the patients have a prolonged bleeding time but a normal number of platelets, one might suspect a defect in platelet adhesiveness In thrombasthenia absence of aggregation of the platelets with ADP and absence of adhesiveness to glass have been found by various authors using many different techniques (7, 8 26) In von Willebrand's disease, however all methods devised include measuring the adhesiveness and aggregation under circumstances where very small changes in the procedure will cause disproportionally large differences in the results Thus sampling procedure, concentrations of ADP and citrate character and length of the filters the rate of flow, time interval between blood sampling and passage through the filter, temperature and inadequacies at the platelet countings are all factors influencing the results

It is also imperative that the diagnosis be correct This means that the patients should fulfil the criterica already mentioned i e a prolonged Ivy bleeding time decreased AHF and a typical hereditary pattern Since it has not been shown with certainty that the platelet adhesiveness is constantly low in von Willebrand's disease, it is of course not warranted to use such a decrease *alone* as a criterion of the disease

Strauss and Bloom (25), for example, recently reported a study on platelet adhesiveness, as measured by Salzman's method, in patients with von Willebrand's disease They used a low platelet adhesiveness as the most important diagnostic criterion of the disease Some of their patients evidently had von Willebrand's disease, but most of them did not fulfil the diagnostic criteria Thus, many cases were sporadic and others had various modes of inheritance Persons having no bleeding symptoms, normal bleeding time and normal AHF, but a low platelet adhesiveness as the only abnormality were considered to be carriers of the von Willebrand gene No correlation was found between prolonged bleeding time and low platelet adhesiveness

As to the various methods applied, Vainer and Caen (26) and later Hellem and co workers (28) used platelet rich citrated plasma with addition of ADP These methods have the advantage that they are not affected by the quality of the red cells, but the disadvantage that ADP will induce a very high aggregation and adhesion at 0 3 μg/ml plasma and hardly any at 0 02 μg/ml From the theoretical point of view it would be preferable to use ADP in excess to prevent moderate changes in the concentration from interfering with the results Unfortunately, however only patients with thrombasthenia will show abnormal adhesiveness at high concentrations It was therefore necessary to use a low concentration of ADP about 0 05 μg/ml, but at this level small changes in the ADP concentration will produce disproportionately large changes in the platelet adhesiveness The method is further complicated by the fact that ADP is rapidly destroyed in platelet rich plasma and that this breakdown is influenced by temperature and various enzymes Thus, the results depend not only on the actual platelet adhesiveness but also on the rate of ADP degradation

The whole blood method of Hellem with citrated blood does not involve the risk of ADP breakdown, but the number and quality of the red cells will influence the results, and the addition of citrate implies the addition of an agent which at higher concentrations will completely abolish the adhesiveness In his method, however, the rate of flow is strictly standardized

In our own experiments we have examined 68 patients with von Willebrand's disease As we had investigated 57 patients with the ADP plasma method of Hellem and co workers (28) but found no difference in the adhesiveness, we were invited to Oslo with five of our Swedish patients to find out why we got different results At this investigation, however, the Norwegians could not confirm their preliminary results We have also investigated the platelet adhesiveness in citrated whole blood in 67 patients and found no difference Nor have we found any difference between mild and severe cases or found any change after treatment with fraction 1—0 The platelet rich plasma reacted with platelet aggregation in a normal way after addition of collagen

After having investigated the platelet adhesiveness in citrated whole blood and in plasma after addition of ADP

in a large material of typical Swedish patients with von Willebrand s disease, we must conclude that there is no defect in platelet adhesiveness to glass or any abnormal reaction to ADP or collagen

Summary

Platelet adhesiveness in citrated whole blood and in plasma after addition of ADP has been studied in 68 Swedish patients with von Willebrand s disease No decrease in platelet adhesiveness was found with either method

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From Medical Service I University of Goteborg Goteborg Sweden

Haemodynamic Effects of Digitalis in Acute Myocardial Infarction

By

RAOUL MALMCRONA, GUSTAV SCHRODER and LARS WERKO

Digitalis has been abandoned by many doctors as an agent for the treatment of patients with acute myocardial infarction on account of its toxic actions which were supposed to be especially dangerous when the myocardium was damaged However, after treating 50 patients with acute myocardial infarction with digitoxin Askey (1) concluded that the fear of catastrophic arrhythmias was exaggerated Then Boyer (3) reported a favourable effect on the course of the illness and later Sanazaro (10), Biné (2) and Willems and Schuller (13) recommended use of digitalis with the same indications in patients with myocardial infarction as for other patients e g heart failure and certain arrhythmias Recently Cronin and Zsoter (4) reported a favourable effect of digitalization in experimental cardiogenic shock

The circulatory derangement in myocardial infarction is complex and the optimum treatment is not yet established In order to clarify the problem the specific haemodynamic effects of the available drugs need to be shown

This paper deals with the effect of digitalis on the systemic circulation in a defined group of patients with acute myocardial infarction All were males They had a transmural infarction most had a substantial fall in brachial arterial pressure but none had left ventricular failure or cardiogenic shock All were in sinus rhythm when studied

Material

Ten patients aged 45 to 77 years were studied The study was performed within four days of onset of symptoms

All patients had pains referable to the onset of myocardial infarction all had a temperature reaction all had a rise in serum glutamic oxaloacetic transaminase and all had electrocardiographic signs of transmural infarction

Six patients had a substantial fall in arterial blood pressure in the first few days of hospitalization but only one had a systolic pressure below 100 mm Hg at the time of the study None of the patients had symptoms or physical signs of left ventricular failure when studied An X ray examination of surviving patients before they left the hospital disclosed an increased heart size in

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TABLE I Clinical data for patients digitalized after recent myocardial infarction

Patient	Age (yrs)	Height (cm)	Weight (kg)	Previous related disease	No of days with rectal morning temperature above 37 C and 38 C respectively	Glutamic oxaloacetic, transaminase (units)	Blood pressure levels (mm Hg)	Location of infarct electrocardiographically
1	45	182	87	None	6–2	316	120/80 stable	Diaphragmatic
2	65	186	94	None	11–3	206	120/80 stable	Anterior
3	74	174	68	Hypertension 20 years myocardial infarction 11 years ago	2–0	670	130/70 60/– 100/–	Anterior
4	67	169	72	Hypertension and effort angina 1 year	10–4	430	180/110 95/– 120/80	Anterior
5	59	167	71	None	1–0	460	160/90 120/80	Anterior
6	58	179	68	None	3–2	14 700	190/130 80/– 120/80	Anterior
7	58	172	67	None	10–2	185	160/100 95/65 110/70	Diaphragmatic
8	77	164	71	None	6–2	175	160/100 90/50 115/80	Diaphragmatic
9	59	184	64	None	7–4	180	105/65 100/70 115/70	Anterior
10	63	172	66	Diabetes mellitus 6 years effort angina 6 months	5–0	124	140/80 120 80 130/70	Diaphragmatic

Rhythm	Day of st[illegible] ly fr[illegible] ct of symptoms	Morning temperature [illegible] day [illegible] °C	State [illegible] digitalization	Heart size [illegible] X-ray [illegible]	Subsequent course
SR	3rd	38.4	Unremarkable	Increased	Returned to work after one year
SR VES 1st day BBB from 2nd day	2nd	38.2	Unremarkable	Increased	Died on 19th day. Total occlusion of desc. branch of left cor. art.
SR BBB atrial fibrillation and idioventricular rhythm 3rd day	2nd	37.7	Unremarkable	Increased	Died on 3rd day. Almost total occlusion of desc. branch of left cor. art.
SR BBB from 2nd day	2nd	38.7	Pains and cold sweat	Normal	Died on 10th day. Total occlusion of right cor. art. and desc. branch of left cor. art.
SR	3rd	37.5	Unremarkable	Normal	Returned to work after [illegible] months. Died in recurrent infarction 6 months later. Total occlusion of desc. branch of left cor. art.
SR AV II and AV III from 3rd day	1st	37.7	Pains	Normal	Died on 3rd day. Total occlusion of desc. branch of left cor. art.
SR	2nd	39.6	Pains and cold sweat	Normal	Returned to work after [illegible] months
SR VES 1st day	3rd	38.[illegible]	Unremarkable	Increased	Died in recurrent infarction after 8 months. At autopsy an old posterior and a recent anterior infarction were seen
SR supraventricular extrasystoles	4th	39.0	Unremarkable	Normal	Returned to work after [illegible] months. Recurrent infarction 1 year later. Again returned to work
SR	3rd	38.0	Unremarkable	Normal	Returned to work after [illegible] months

SR sinus rhythm
VES ventricular extrasystoles
BBB bundle branch block
AV atrioventricular block

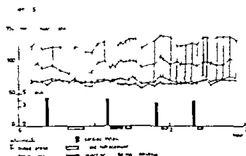


Fig 1 Diagram showing procedure and results in one of the patients Heart rate blood pressure and cardiac output are given and injection of lanatosid C and blood replacements indicated

two patients They had also signs of pulmonary congestion

Two patients had atrioventricular heart block recorded the day after and two days after the investigation respectively Bundle branch block was seen in three patients and ventricular extrasystoles in two patients None of these arrhythmias was referable to the digitalis injection

Four patients died during the hospital stay Two patients have died from recurrent infarctions 8 and 12 months after their first one

A summary of clinical data is given in table I

Methods

The studies were performed in the ward with the patient lying in bed Catheters were inserted percutaneously into the brachial artery and an antecubital vein The venous catheter was advanced centrally ECG and arterial pressures were recorded with an ink jet writing oscillograph (Elema Co)

TABLE II Haemodynamic data for patients with acute myocardial infarction given 0 8 mg lanatosid C

	Control						
				Brachial arterial pressure			
Patient	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)
1	73	6 0	82	105	69	83	14
	75	5 3	71	106	70	84	16
2	112	7 3	65	116	62	81	11
	112	7 0	63	109	60	78	11
3	82	4 3	52	110	73	87	20
	82	4 1	50	104	67	80	20
4	78	6 1	78	117	68	90	15
	85	6 2	73	112	62	81	13
5	65	4 4	68	121	70	90	20
	71	4 2	59	132	73	102	24
6	94	6 1	65	141	92	111	18
	95	4 5	47	130	84	105	23
7	91	6 0	66	105	56	75	13
	96	5 9	61	109	57	75	13
8	59	5 8	98	127	53	80	14
	57	5 2	91	119	52	80	15
9	—	—	—	—	—	—	—
	110	5 4	49	88	50	66	12
10	—	—	—	—		—	—
	84	6 6	79	122	59	84	13

The pressures were transferred by a transducer of the variable inductance type (Elema Co) Mean pressures were obtained by electrical integration Reference point was 5 cm below the sternal angle Cardiac output was determined by a dye-dilution technique with use of bromsulphalein as indicator (11) Total peripheral resistance was calculated by dividing mean brachial arterial pressure expressed in mm of Hg by cardiac output expressed in l/min

Means standard deviations and standard errors of the means were calculated The probability of a difference was tested by Student's t test and differences at or below the 5 per cent level were considered significant

ECG and brachial arterial pressures were recorded every 2—5 minutees When the heart rate and the pressure were stable and when at least 30 minutes had passed after the insertion of the catheters a cardiac output determination was made The recordings of heart rate and pressure were continued and about 20 minutes later a second cardiac output determination was made in eight patients The patients were then given 0 8 mg lanatosid C intravenously over 10 minutes Thirty and 60 minutes later the cardiac output determinations were repeated After every cardiac output determination the blood loss of about 40 ml was substituted for by stored blood

The procedure is shown diagrammatically in the figure, where results of the investigation in one of the patients (patient 5) are graphed

The two studies performed before digitalis was given were compared so as to evaluate the spontaneous variation in each patient

Control values 30 minutes and immediately before the drug and values 30 and 60 minutes afterwards

After lanatosid C

Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Brachial arterial pressure Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)
73	6 2	85	110	68	86	14
76	6 1	80	111	69	86	14
110	7 7	70	116	62	82	11
113	7 6	67	116	62	83	11
80	3 9	49	118	73	98	25
70	3 4	49	107	67	81	24
86	7 0	81	117	62	89	13
91	7 6	84	109	58	84	11
66	3 7	56	134	69	97	26
69	4 0	58	130	70	98	24
92	5 4	59	148	86	114	21
93	4 8	52	146	88	111	2[illegible]
92	6 0	65	109	64	84	14
86	6 0	70	111	65	86	14
58	4 9	84	137	55	81	17
64	5 8	91	130	52	81	14
111	5 1	46	100	57	71	14
114	5 2	46	94	53	66	13
88	6 7	76	125	54	82	12
89	6 7	75	122	56	84	11

TABLE III Mean haemodynamic data for patients with acute myocardial infarction given 0 8 mg significance of differences of values recorded 30 minutes before giving the drug (first minutes afterwards (60 minute result) are given

		Heart rate (beats/min)	Cardiac output (l/min)
First control	M±SE	82±6	5 8±0 3
n 8	SD	17	1 0
Second control	M±SE	87±5	5 4±0 3
n 10	SD	17	1 0
Difference between first and	M±SE	−2±1	−0 5±0 2
second control	SD	4	0 5
n−8	P	—	<0 05
30 minute result	M±SE	86±5	5 7±0 4
n=10	SD	17	1 3
Difference between second	M±SE	-1±1	+0 2±0 2
control and 30 minute result	SD	3	0 6
n=10	P	—	—
60 minute result	M±SE	87±6	5 7±0 4
	SD	17	1 4
Difference between second	M±SE	±0±2	+0 3±0 2
control and 60 minute result	SD	6	0 6
n=10	P	—	—

The studies made 30 and 60 minutes after digitalis had been given were both compared with the study made just before giving digitalis

Results

Haemodynamic data for each patient are given in table II and mean values in table III

Heart rate

Mean heart rate was unchanged in the control period as well as after digitalis had been given The maximal individual changes were decreases of 10 and 12 beats respectively *in two patients 60* minutes after digitalis had been given

Cardiac output

No extreme values of cardiac output were encountered

Cardiac output decreased in the control period The maximal individual change was a decrease of 1 6 l min concomitant with a fall in brachial arterial pressure There was no significant change after digitalis had been given

Stroke volume

Stroke volume also decreased in the control period but there was no change in the mean value after digitalis had *been given The maximal individual* changes were increases of 14 and 12 ml

lanatosid C Mean values (M) standard error (SE) and standard deviation (SD) differences and control) immediately before (second control) 30 minutes afterwards (30 minute result) and 60

Stroke volume (ml)	Brachial arterial pressure Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	Resistance (units)
72±5	118±4	68±4	87±4	16±1
14	12	12	11	3
64±5	113±4	63±3	84±4	16±2
14	13	10	12	5
−6±1	−3±2	−2±1	−2±2	+1±1
3	7	4	7	2
>0 001	—	—	—	—
67±5	121±5	65±3	88±4	17±2
14	15	10	12	5
+3±2	+8±2	+2±1	+5±2	−1±1
7	7	4	7	2
—	<0 01	—	~0 05	—
67±5	118±5	64±3	85±4	16±2
16	15	11	12	6
+3±2	+5±2	+1±1	+3±1	±0±1
6	6	4	4	2
—	<0 05	—	—	—

respectively in two patients 30 minutes after digitalis had been given

Arterial pressure

Mean systolic pressure was unchanged in the control period During the 10-minute injection of lanatosid C the pressure rose in all patients This higher pressure was still found at 30 as well as at 60 minutes after injection The maximal individual changes were an increase of 22 mm Hg in one patient in the control period and increases of 18 in two and 16 mm Hg in one patient 30 minutes after injection

The brachial mean pressure was unchanged in the control period and higher 30 minutes after digitalis had been given

Brachial diastolic pressure was unchanged in the control period as well as when digitalis had been given

Total peripheral resistance

The resistance was unchanged in the control period as well as when digitalis had been given

Discussion

The patients with acute myocardial infarction were selected from a larger series of patients (7) to constitute a more homogeneous group suitable for pharmaco-therapeutic study

The digitalis preparation lanatosid C is extensively used for intravenous thera

the presence of ε ACA In the tables and figures we have therefore only given one fibrinogen value

Platelet factors Platelet suspensions were studied in respect to the following platelet functions (1) platelet factor 1 (= factor V activity) (2) platelet factor 3, (3) platelet factor 4 (heparin binding capacity) (29)

Platelet adhesiveness This was measured by a slight modification of the method of Hellem (17) as described by Cronberg et al (11)

Assays for components of the fibrinolytic system The blood was assayed for fibrinolytic activity by determination of (a) plasma euglobulin clot lysis time, (b) the activity manifested by plasma and resuspended euglobulin precipitate put on unheated and heated fibrin plates and (c) whole blood clot lysis These determinations were performed in accordance with Nilsson and Olow (28)

The activity of plasminogen and pro activator in citrated plasma was determined by the clot method described by Nilsson et al (30)

Serum inhibitors of plasminogen activation by urokinase were measured according to Paraskevas et al (34) The *antiplasmin* activity of serum was determined according to Nilsson et al (26)

The inhibiting effect of serum on tissue activator from pig heart (anti activator assay) was determined as described by Nilsson et al (23)

The *trypsin inhibitory effect* of serum was assayed by incubation with a solution of trypsin and determining the degree of inhibition produced The trypsin activity was determined by 1) a caseinolytic method (16) and 2) by the heated fibrin plate method in the manner described by Nilsson et al (26)

Paper electrophoresis Paper electrophoresis of 25 mm³ citrated plasma was performed according to the technique described by Bielawiec and Nilsson (4)

Fibrinolytic split products Plasma and serum were investigated before and after activation with streptokinase for the presence of fibrinolytic split products by means of the immunological method described by Niléhn and Nilsson (22)

Fibrin stabilizing factor (*f XIII*) was determined as described by Duckert (13)

Case report

The patient was *born in 1929* Foster-child Parents unknown No brothers or sisters Normally developed No malformations As a child he had had measles and whooping cough

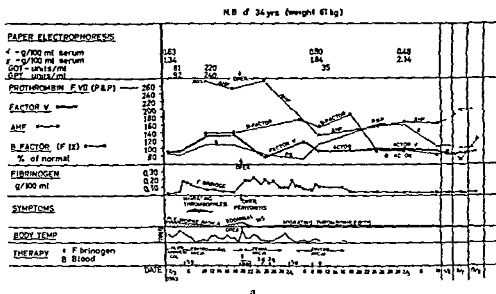
In *1938* the patient was admitted to hospital because of acute appendicitis with peritonitis The post-operative course was complicated by right sided pleurisy and high grade fever Haemorrhagic pleural fluid was tapped on 3 occasions Clinically clear thrombosis of the left leg supervened as well as transient gross haematuria Urography revelaed nothing remarkable Erythrocyte sedimentation rate (Westergren) 0—6 mm/l hour

In *1952* the patient then being 23 obstinate sores appeared on the left leg

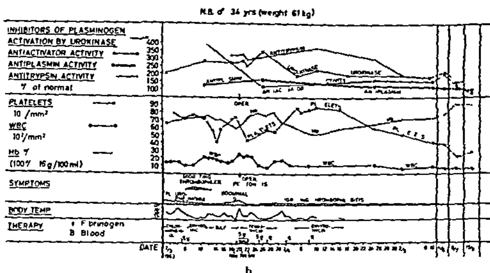
In *1957* a blow against the left lower leg was followed by signs of thrombosis at the site of the injury Phlebography revealed an old thrombosis extending up into the iliac vein and acute thrombosis of the lower leg and possibly also of the recanalised femoral vein *Laboratory studies* E S R 1—2 mm/l hour Hb 14 6 g/100 ml WBC 6,200 per mm³ Differential count normal Platelet count 199 000 per mm³ Fibrinogen 0 02—0 04 g/100 ml Liver biopsy revelaed nothing remarkable The patient bruised readily and had small haematomas on the trunk and limbs

He afterwards felt well until *1961* when he was admitted for tooth extraction under cover of fibrinogen He received altogether 23 g of fibrinogen (fig 3) The course was initially uncomplicated Three months after treatment however serum inoculation hepatitis developed and persisted for 2 months

In *1962* he complained of pain and a feeling of tension in the left big toe which was cyanotic In the autumn of that year pain developed in the right leg and periodically



a

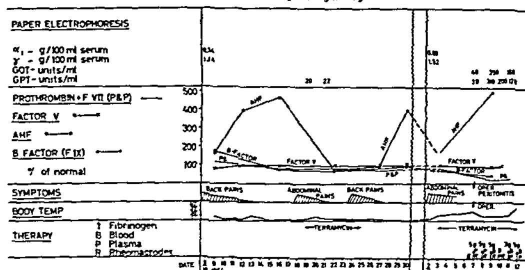


b

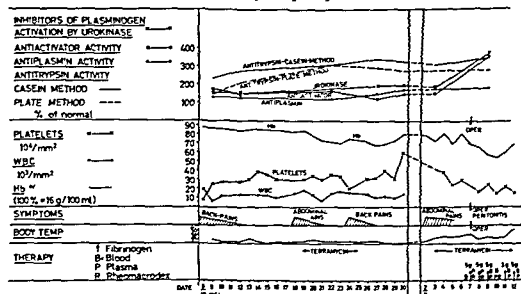
Fig 1 a and b Ileotomy (episode I) Treatment laboratory data and course

in the right side of the back Neither roentgen examination of the colon chest and lumbar region nor urography revelaed anything remarkable Laboratory data were also normal

In February 1963 the patient complained of acute pain in the left flank Clinical examination on admission to hospital revealed nothing of interest There were no signs of increased bleeding tendency Urography and roentgen examination of the chest and lumbar spine showed nothing remarkable Chest X ray revealed atelectatic changes in the base of the left lung and slight left sided pleural effusion High grade fever was recorded E S R 3 mm/1 hour WBC 14 000 per



a



b

Fig 2 a and b Ileotomy (episode II) Treatment laboratory data and course

mm³ Platelet count 648 000 per mm³ Peritonitis developed At operation under cover of fibrinogen the ileal loop was found to be gangrenous and perforated. During the post-operative course subcutaneous thrombophlebitis appeared in the abdominal wall and arms and the platelet count increased to 1 million Laboratory data are given in figs 1 a and b The patient returned to work as a clerk 2 months later

The patient felt well until *October 1964* when after a prolonged, obstinate respiratory tract infection he again complained of severe abdominal pain particularly in the left flank Examination revealed high grade fever and leukocytosis Urography showed nothing abnormal

The clinical picture gradually became alarming and peritonitis was assumed Under protection of fibrinogen 2 metres of gangrenous small intestine was excised Initially after the operation the patient appeared to improve but 2 days later he suddenly went into shock and died Laboratory data are given in figs 2 a and b

N.B ♂ 32 yrs (weight 70 kg)

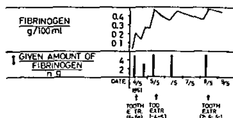


Fig 3 Tooth extractions under cover of fibrinogen

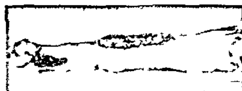


Fig 4 A 12 cm long thrombus in the lower thoracic aorta and upper abdominal aorta

Pathological examinations

March 1963 Operative specimen of ileum Medium sized arteries in the mesenterium contained thrombi with incipient fibroblastic organisation A thrombus entirely invaded by fibroblasts was seen in the submucosa beneath well preserved mucosa The intestine showed a necrotic area with a perforation surrounded by mucosal necrosis and in other layers of the wall haemorrhagic granulation tissue with numerous neutrophils and eosinophilic leukocytes fibroblasts and some round cells Orally and aborally to this lesion the oedema and inflammatory changes in the submucosa decreased as the subserosa with granulation tissue became thicker The mesenterium showed leukocytes abscesses and fibroblasts

November 1964 Operative specimen of ileum and colon In the mesenterium and the walls of the ileum and colon the arteries and veins contained numerous partly organised partly recent thrombi which were hyaline or showed strands with the appearance of fibrin Numerous leukocytes and partial or complete haemorrhagic necrosis were seen in the walls of some vessels, especially veins Thrombi and haemorrhagic foci were seen also in blood vessels and lymph nodes In the ileum there were widespread mucosal necroses and haemorrhages in the submucosa

Calcified lymph nodes were seen in the ileo-coecal angle The caecum and the ascending colon showed submucosal fibrosis and in some areas recent haemorrhagic mucosal necrosis

No signs of actual specific inflammation or regional enteritis

November 1964 Post mortem examination The findings were dominated by multiple old and recent thrombi The lower thoracic aorta and upper abdominal aorta contained a 12 cm long thrombus the thickness of a finger (fig 4) It was adherent to the wall and consisted of a fresh thrombus with a fibrin net and aggregated red blood cells superimposed on an old organised thrombus with numerous capillaries in an acellular partly necrotic connective tissue The inner surface of the media was also partly necrotic with round cell and leukocyte infiltrates partly fibrotic with collagen In the lower abdominal aorta there was a thrombotic plate some millimetres thick and adhering to the wall Recent and older thrombi in all stages of fibrous organisation partly recanalised and harbouring fresh thrombi were also seen in the main trunk of the hepatic artery and its large branches in intrahepatic portal branches in the splenic artery and vein with recent and old splenic infarctions in the

TABLE I Results of coagulation studies

Test	19/8 1957	31/10 1957	11/3 1958	25/2 1959
Platelets per mm³	221 000			234 000
Prothrombin + f VII + f X (P & P) %	82	79	87	140
Factor V %	50	100	90	130
AHF (f VIII) %	30	80	>100	185
Haemophilia B-factor (f IX) %				68
Fibrinogen g/100 ml	0 04	0 06	0 06	0 07
Fibrinolytic activity on unheated fibrin plates mm²				
a Plasma	Pos	Neg	Neg	54
b Resuspend euglob prec				
Plasminogen+proactivator activity %	83	93	110	104
Inhibitors of plasminogen activation by				
1 Urokinase %				
2 Streptokinase %				50
Antiplasmin activity %			100	100
Antiactivator activity %				
Trypsin inhibitor γ trypsin inactivated by 1 ml serum			623	
Thrombin time (3 NIH units/ml) sec	44 (normal=21)			

superior mesenteric artery and vein and in their branches right out into the submucosa Widespread infarction was also observed in the residual small intestine partly with abundant leukocytes fibrin-coated serosa and almost 2 litres of brownish cloudy ascitic fluid Hyaline or fibrous organised thrombi were seen in the small and medium sized arteries in the kidneys with multiple old and recent infarctions The lungs showed numerous fresh free masses of thrombi in the medium sized vessels and hyaline thrombi less recent haemorrhages and brown pigmented phagocytes in the smaller vessels

Apart from some lipid plaques in the coronary vessels aorta and carotid vessels no arteriosclerotic changes were noted Besides the aorta mesenteric vessels displayed inflammatory infiltration with many thrombosed vessels showing no degenerative or inflammatory changes There was no evidence of generalised vascular disease

Results of coagulation studies (table I and figs 1 a and b, 2 a and b and 3)

The patient was examined at least 100 times between 1957 and 1964 At all examinations the fibrinogen level was low (range 0 02—0 07 g/100 ml) except for those studies performed during fibrinogen therapy Even administration of ε ACA for 7 days in a dose of 7 g four times daily (all together 182 g) did not change the low fibrinogen value Only on one occasion in August 1957 when the patient had widespread thrombosis in his left leg was the fibrinolytic activity

31/10 1960	8/11 1960					
1/11—8/11 ε ACA 7 g × 4 = 182 g		26/11 1962	1/3 1963	17/12 1963	20/7 1964	3/11 1964
204 000			648 000	218 000	194 000	
69	97		91	116	112	86
90	66		94	98	92	95
189	180			202	152	158
57	59			128	70	78
0 05	0 05	0 03	0 03	0 06	0 04	0 05
0	0	0	0	0	0	0
26	—	68	127	47	0	18
80			137	108	140	107
120		182	200	190	202	190
100		89	168	128	—	
150		107		94	69	171
100		68		170	104	149
				880		1 770
			52	20	23	22

slightly increased in all other investigations no activity could be demonstrated In connection with the fibrinolytic activity the AHF and factor V level decreased but in all other analyses these factors were normal or even increased especially when the patient was operated upon The coagulation time the one stage prothrombin time and the thrombin time were always prolonged except when the patient had received fibrinogen when these times were normalized The prothrombin and factor VII value (P & P) and factor IX (haemophilia B factor) were normal The plasminogen and pro-activator activity was normal The inhibitors of the plasminogen activation by urokinase and streptokinase were normal The antiplasmin activity the anti activator activity and the trypsin inhibitors were also found to be normal The platelet count and the bleeding time were normal

When the patient had infections and was operated upon the platelet count, the AHF and the inhibitors of plasminogen activation by urokinase and the trypsin inhibitors increased to very high values The values for haemophilia B-factor prothrombin and factor VII and V also increased but not to such high values (fig 1 a and b and 2 a and b) No fibrinolytic activity could be demonstrated in the post-operative course Around the time of the operation in April 1963 he did not show normal values for the various coagulation factors the platelet count and the fibrinolytic inhibitors until 4 months after he had become ill

The platelet factors 1 3 and 4 were all normal.

Fibrin stabilizing factor was normal

Paper electrophoresis showed at the site of the *fibrinogen* a narrow band representing 0 05 g/100 ml of the total plasma protein (6 8 g/100 ml) whether the electrophoresis was done without or with ε ACA in the buffer

Fraction I O was prepared from the patient s plasma according to the glycine method of Blombäck and Blombäck (5) From 100 ml plasma a fraction was obtained which contained about 0 04 g protein i e about 25 % of the amount obtained from normal plasma The clottability of this fraction was the same as that found for corresponding fraction obtained from normal plasma Fraction I O from the patient and a normal person showed the same precipitation arcs on immunoelectrophoresis against rabbit anti human I O serum The fibrinogen arc was fainter than for normal fraction I O but it was situated at the same site

The values obtained on determination of the patient s fibrinogen content by an immunological method (33) agreed with those obtained by the precipitation method with thrombin

On activation of the patient s plasma with urokinase and streptokinase respectively, plasmin activity was obtained and the patient s fibrinogen was broken down The fibrinogenolytic degradation products were studied by immunoelectrophoresis (22) and the final products, i e D and E products (31), which are normally formed were demonstrated also in the patient s plasma

No cryoglobulin (dissolvable at 37° C) was demonstrable in the patient s plasma or serum

The patient s *fibrinogen was precipitated* normally when the plasma was heated to 56° C. No gel formation was noted

Estimation of the adhesiveness of the platelets In February, July and August 1963 the platelet adhesiveness in citrated whole blood was normal namely 25 % The platelet count was also normal except on the first occasion when it was 810 000 per mm³

In October 1964 the platelet adhesiveness in citrated whole blood was repeatedly found to be about 47 % The platelet adhesiveness in citrated platelet rich plasma after addition of ADP proved normal

The patient s platelets underwent normal viscous metamorphosis after recalcification of platelet rich plasma The platelets aggregated normally after addition of ADP collagen and thrombin

The clot retraction appeared normal

Discussion

We have followed this patient regularly since 1957, i e for 7 years, and made determinations of fibrinogen on at least 100 occasions, often when the patient was symptom free The fibrinogen value was always in the range 0 02—0 07 g per 100 ml The first blood samples collected in the autumn of 1957 showed a slightly increased fibrinolytic activity He then had venous thrombosis of the left leg From 1958 on, however, the fibrinolytic activity was not increased and the fibrinogen content remained unchanged In the course of a week he was given ε ACA in a total dose of 182 g The fibrinogen content remained unchanged In 1961 the patient received altogether 23 g of fibrinogen in association with tooth extractions The fibrinogen was metabolised at a normal rate (fig 3), and no products of fibrinolysis were demonstrable in the patient s serum Thus, nothing suggested that the decreased fibrinogen content was due to an increased fibrinolytic activity

In the autumn of 1957 the increased fibrinolytic activity was found to be accompanied by decrease of AHF and of factor V, which was ascribed to the increased fibrinolytic activity Coagulation analysis during t ears 1958—1962 s[illegible] platelet

count was also normal. This argues against the patient's low fibrinogen being due to continuous intravascular coagulation.

The fact that the fibrinogen value was constantly low during the entire study period suggests that he might have had congenital hypofibrinogenaemia. It is true that the fibrinogen had not been measured previously, but records of the spells he spent in hospital during childhood show that the E.S.R. was not increased despite high grade fever, pneumonia and peritonitis. Unfortunately the diagnosis could not be confirmed by examination of his parents and other relatives, for his parents were unknown and he himself had no children.

It is also possible that the low fibrinogen value might have been due not to a true decrease of the fibrinogen content, but to a normal amount of an abnormal fibrinogen whose coagulability was only about 20 % of that of normal fibrinogen. But the patient's fibrinogen migrated at a normal rate in the electrophoretic field, it was precipitated in the normal way when heated at 56° C and was precipitated also in the usual way on alcoholic fractionation of the plasma. The yield of protein obtained in these procedures was small and corresponded to the calculated amount of fibrinogen. No cryoglobulin could be demonstrated in the patient's plasma or serum. The patient's fibrinogen and fibrin was broken down normally by plasmin with formation of fibrinogen degradation products with normal antigenic determinants. Quantitative immunological determination of the fibrinogen content in the plasma from the patient gave a low value corresponding to that obtained on precipitation with thrombin. Peptide A and B were admittedly not determined. Nothing suggested that the patient had abnormal fibrinogen of the type described by Imperato and Dettori (18), Menache (21), Beck et al. 3.

In 1957 the patient had one episode with increased bleeding tendency during a period with increased fibrinolytic activity. Otherwise he showed no bleeding tendency whatsoever. The remarkable feature of this case is the increased tendency to thrombosis. Other published cases of congenital hypofibrinogenaemia are described as having had slightly increased bleeding tendency, particularly in association with surgical intervention. In only one case was the hypofibrinogenaemia seen in association with massive thrombosis 20. These authors suggested that according to the theory of Copley (10) the disappearance of a *protective film* of fibrin which covers the vascular endothelium might be responsible for this curious complication of hypofibrinogenaemia. Caen et al. (8) have described bilateral ischaemic necroses in the leg in one case of congenital hypofibrinogenaemia. They could not explain the mechanism of these changes but like Marchal and coworkers (20), they assumed that deficiency of a vascular fibrin film was responsible.

We suspected that the fibrinogen given might have had an undesirable effect. However already at 9 years of age the patient had had widespread thrombosis and in 1957 also a recurrence of thrombosis that had not been preceded by administration of fibrinogen or blood.

The fibrinogen that was given in 1961, in association with tooth extraction, was consumed at a normal rate There is therefore no reason to suppose that this fibrinogen had produced thrombosis On the last two occasions when the patient was given fibrinogen in association with the operations, the fibrinogen was consumed rapidly without any signs of *fibrinolysis, and it is* probable that this fibrinogen had at least contributed to increase the then existing thrombosis On the other hand, it is obvious that when the patient had thrombosis he also had high grade fever and laboratory studies showed a pronounced reactive process The platelet count, the AHF content and the various fibrinolytic inhibitors then increased to higher values such as we believe are usually seen in connection with a reactive process (2, 14, 32) (figs 1 a and b, 2 a and b)

No explanation can be offered for the increased tendency to thrombosis and the formation of the massive thrombosis also in the large vessels in this case We feel however that the increased tendency might be a consequence of the "hyperactive values" in association with the reactive process The small thrombi that are formed are not dissolved by fibrinolysis, since no spontaneous fibrinolytic activity was demonstrable and the values noted for the inhibitors of fibrinolysis were high and the small thrombi formed provided a platform for superposition of further deposits of platelets According to Poole (36) a white thrombus consists of agglutinated platelets and is relatively low in red blood cells and fibrin Johnson (19) points out that this holds especially for thrombi in the arteries, where the very fast blood stream carries activated plasma coagulation constituents downstream as fast as they can form and consequently no fibrin network can be formed At pathological examination it is impossible to differentiate between platelets and fibrin in the thrombus This patient, who always had a *low fibrinogen* value *but* none the less developed multiple large venous and arterial thrombosis, may therefore illustrate the important role of platelets in thrombus formation

How should this patient have been treated? It might be argued that it would have been better not to have given the patient fibrinogen during the operations It was, however, considered contraindicated to perform a major operation on a patient with such a low fibrinogen value as 0 04 g/100 ml The fibrinogen given had most likely increased the size of the thrombi formed during the operations, but as pointed out above, it cannot have been the precipitating factor One might also wonder whether it might not have been advantageous to give heparin Heparin would not have reduced platelet adhesiveness but it would have inhibited the subsequent phases of coagulation Treatment with streptokinase would in this case perhaps have been of value It was, however, believed that the risk of bleeding after extensive operations was too great to allow such treatment

Summary

A man with probably congenital hypofibrinogenaemia, who since early childhood had had a pronounced predisposi

tion to thrombosis and thrombophlebitis and who died at the age of 36 with widespread venous and arterial thrombosis, also in the large vessels The fibrinogen level was 0 04 g/100 ml determined by precipitation with thrombin, alcohol fractionation, electrophoresis and by immunological technique Nothing suggested the presence of an abnormal fibrinogen, and the survival time of injected fibrinogen was normal The platelets and the various coagulation factors were normal except when the patient had infections or was operated upon, when the platelet count, the AHF content and the various fibrinolytic inhibitors increased to very high values In connection with such episodes thrombosis and superficial thrombophlebitis developed

Acknowledgement

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From Medical Department E (Heads N I Nissen and Th Friis) and the Central Laboratory (Heads A Levin Nielsen and J Date) Frederiksberg Hospital Copenhagen Denmark

Effect of Methandienone (Methyl Androstenolone, 1-Dehydro-17 α-Methyl Testosterone) (Geabol, Dianabol) on Pituitary Function

By

TH FRIIS and S SPARREVOHN

Since about 1950 the synthesis has been achieved of many steroids having a pre dominantly anabolic action The most important group of these agents has been that of the 17 α alkyl substituted testosterone derivatives The literature on the effect of the anabolic stereoids upon endocrine glands is limited Wynn et al (13, 34) demonstrated that in normal persons and in patients with endocrine disorders the anabolic steroid methandienone (Geabol, Dianabol), in daily doses of 10—100 mg inhibits release of ACTH from the pituitary This manifested itself int al in an abnormal metyrapone test and a reduced urinary excretion of 17 ketosteroids (17 KS) and 17 hydroxy corticosteroids (17 OHCS) Wynn et al believed that the suppression was due partly to a reduced breakdown of corti sol in the liver caused by the special hepatic action of the drug and partly by a direct suppressive action upon the hypothalamus hypophysis Others (1 12 24) have reported that testosterone or methyl testosterone (Nilevar) may reduce the urinary excretion of corticosteroids However there is disagreement concerning these alterations in steroid excretion, as several authors (22 23, 28) have been unable to confirm the finding

It has been demonstrated that methyl-testosterone reduces the binding of thyroid hormone to plasma proteins This leads to an increased amount of unbound hormone in the blood which again may cause an increased suppression of pituitary secretion of thyroid stimulating hor mone and as a result thyroid function is suppressed (5) Rosenberg et al (25) found 17 α ethyl 19 NOR testosterone (Nilevar) and methandienone (Dianabol Geabol) to lower protein bound iodine (PBI) and to increase the triiodothyronine uptake by ion exchange resin They attributed this to a reduced binding of thyroid hormone to the plasma proteins This can hardly be a result of the andro-

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TABLE I Sex, age and diagnosis of the patients

Case no	Sex	Age	Clinical diagnosis
I	♀	74	Breast cancer
			Diabetes mellitus
II	♂	62	Macroglobulinaemia
			Duodenal ulcer
III	♀	64	Stroke
			Arteriosclerotic heart disease
IV	♀	54	Duodenal ulcer
V	♀	68	Stroke
VI	♀	43	Duodenal ulcer
VII	♀	61	Duodenal ulcer
VIII	♀	65	Arteriosclerotic heart disease
			Angina pectoris
IX	♂	47	Gastritis
X	♂	35	*Gastritis*
XI	♂	62	Juxtapyloric ulcer
XII	♀	63	Gastric ulcer
XIII	♀	52	Gastric ulcer
XIV	♀	65	Arteriosclerotic heart disease

genic effect of the drugs which is very slight Similarly, 1 dehydro 17 α methyl testosterone (Dianabol), which has only 1/30 the androgenic action of 17 α ethyl 19 NOR testosterone (Nilevar), exerts an equal effect in a dose corresponding to one third of that of Nilevar Olivi et al (21) also demonstrated a reduction in PBI upon administration of anabolic steroid to children

Material and method

Evidently much has yet to be learnt about the effect of anabolic steroids upon the endocrine glands especially the pituitary and thyroid glands We therefore studied 14 patients 4 males and 10 females in the age range 35—74, average 58 years none of whom had known endocrine disorders Prior to administration of methandienone they were subjected to the following investigations

Urinary excretion of 17 ketosteroids (17 KS) (33), 17 ketogenic steroids (17 KGS) (16) and pituitary gonadotropin (14) metyrapone test (250 mg every 2 hours in 24 hours) (19) and fractionated 17 ketosteroids in the urine (15)

Furthermore determination of ^{131}I uptake by the thyroid gland (7) determination of ^{131}I labelled triiodothyronine uptake by erythrocytes (8), determination of free and protein bound ^{131}I labelled triiodothyronine by means of gel filtration (29) — done on 13 patients — and PBI (4) The test for ^{131}I labelled triiodothyronine uptake by the erythrocytes was repeated every 3 or 4 days throughout the period of the study

Serum electrophoresis and determination of fasting fractionated serum lipids were done weekly

Liver function tests were also done weekly by the usual techniques i e glutamic-oxal oacetic transaminases (SGOT) thymol turbidity Takata Ara, alkaline phosphatases and serum bilirubin

On the last 6 patients we also studied the 24 hour blood sugar three times daily for

2 days and did an oral glucose tolerance test after administration of glucose 1 g/kg (not more than 70 g)

Thereafter methandienone 10 mg daily was administered throughout the remainder of the study period After two weeks treatment all the investigations were repeated in the same order while the administration of methandienone was continued

Results

We found that the average spontaneous urinary excretion of 17 ketogenic steroids (17-KGS) showed hardly any change during the administration of methandienone On the other hand the metyrapone test showed in 11 of the 14 patients, a definite inhibition of the pituitary adrenal response Three of these 11 patients then had an intravenous ACTH test which resulted in a normal marked increase in urinary 17-KGS excretion

There was also no fall in 17 KS excretion and no significant changes in the various fractions on determination of fractionated 17 KS in the 24 hour urine

Four patients showed a fall in the urinary excretion of pituitary gonadotropin while in 3 it increased and the remaining 7 showed no changes

Investigation of the ^{131}I uptake by the thyroid gland revealed a fall in the 4 hour uptake in 11 of the 14 patients amounting to from 0 7—32 5 % of the dosage m = 8 9 % of the dosage ± 12 72 (3 out of 11 below the normal limit) The 24 hour uptake showed a fall in 13 of the 14 patients (2 below the normal limit) of from 1 9—34 0 % of the dosage, m = 14 6 % ± 10 91

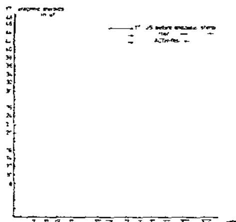


Fig 1 Urinary excretion of 17 ketogenic steroids before and during administration of methandienone The bases of the arrows indicate the excretion prior to administration of metyrapon their tips the maximum 17 KGS value during its administration. It should be mentioned that case VI showed an excessively pronounced increase in the 17 KGS response in the metyrapon test during methandienone treatment. It was this patient who developed nausea vomiting anorexia and general malaise as well as a marked increase in transaminase during the treatment with methandienone Therefore the unexpectedly violent increase in 17 KGS excretion was possibly a manifestation of a stress situation

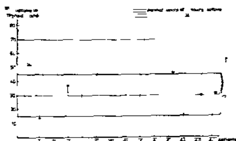


Fig 2 ^{131}I uptake by the thyroid gland before and during administration of methandienone The bases of the arrows indicate the uptake before methandienone treatment and their tips the uptake during the treatment. The solid arrows indicate the 4 hour uptake the broken arrows the 24 hour uptake

The uptake of ^{131}I labelled triiodothyronine by the erythrocytes increased in 13 patients from an average of 0 7 %

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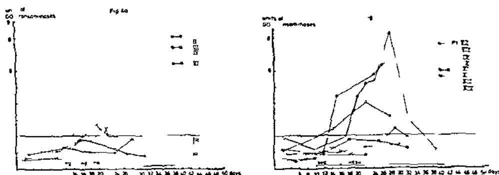


Fig 4 Effect of methandienone upon the serum level of GO transaminases Fig 4 a cases I—VI Fig 4 b cases VII—XIV In patients VI VII and XIV the methandienone administration was stopped when max value was reached

TABLE III Alterations in ¹³¹I uptake by the thyroid in T_3 uptake by the red cells and in GO transaminase during treatment

Case no	Difference in 4 hour ¹³¹I uptake (normal range 15—45%)	Difference in 24 hour ¹³¹I uptake (normal range 30—70%)	Difference in T_3 uptake by erythrocytes (normal range 6 0—10 5%)	Difference in GO transaminase (normal <2 0 units)
1	+ 10 0	− 3 9	+ 0 7	− 0 4
2	3 6	− 16 9	+ 1 4	+ 1 0
3	0 7	− 9 3	+ 2 7	+ 0 6
4	+ 5 0	12 0	+ 5 0	+ 1 9
5	− 20 2	− 1 9	+ 2 7	+ 2 9
6	2 2	− 8 4	+ 3 7	− 4 0
7	− 8 0	− 15 4	+ 2 3	+ 4 2
8	− 9 2	− 25 7	+ 2 7	0 5
9	29 4	− 15 1	− 0 1	+ 0 4
10	+ 5 2	+ 4 6	+ 1 4	0 0
11	− 15 4	− 12 7	+ 2 4	− 2 3
12	− 6 9	− 31 6	+ 1 4	+ 0 7
13	16 7	− 22 0	+ 4 9	0 0
14	32 5	− 34 0	+ 1 9	+ 3 0
	− 135 0	− 204 3	+ 33 1	21 9
Mean	8 9	− 14 6	+ 2 4	+ 1 6
Standard deviation	± 12 72	± 10 91	± 1 43	± 1 40
	0 05>P>0 02	P<0 001	P<0 001	0 01>P>0 001

not exhibit any objective or subjective abnormalities

Table III lists the alterations in the various parameters

The last 6 patients had oral glucose tolerance tests and determination of the 24 hour blood sugar 3 times daily for 2 days These investigations were per

formed before and during the administration of methandienone in order to study its effect upon carbohydrate metabolism The oral glucose tolerance curve altered, in 5 of the 6 patients in a diabetic direction, while in no case did the 24-hour blood sugar levels alter

Fasting, fractionated serum lipids showed an increase in cholesterol in 5 patients, but no definite alterations in the other fractions

Thus, the most marked changes were found in respect of the ^{131}I uptake by the thyroid gland, the uptake of ^{131}I-labelled triiodothyronine by the erythrocytes, the levels of free and protein bound ^{131}I-labelled triiodothyronine measured with use of gel filtration SGO transaminases, and the metyrapone induced increase in 17-KGS excretion (the increase being smaller)

Discussion

Testosterone itself has no effect upon liver function that is demonstrable by clinical or laboratory methods On the other hand, several authors have demonstrated that methyl testosterone and the 17 α alkyl substituted anabolic steroids, e g methandienone and norethandrolone, may affect liver function (2 32) By liver biopsies on patients taking anabolic steroid Schaffner et al (27) showed that 4 out of 27 developed typical cholestasis with bile pigment in the liver cells and in Kupffer s stellate cells According to Hsia et al (11) various anabolic steroids inhibit the transfer of glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to bilirubin They suggested, therefore, that the alterations found in the liver function tests during treatment with anabolic steroids were due to an inhibition of some of the conjugation enzymes in the liver cells However, these findings were not confirmed by Arias (3) who demonstrated in several ways, by several experiments in vivo as well as in vitro, that the conjugation ability of the liver cells remained unchanged during administration of anabolic steroid Arias also found that in hyperbilirubinaemia the bilirubin in the serum remained conjugated In his opinion, therefore, the conjugation took place normally, but the liver cells were unable to secrete the conjugated bilirubin into the bile capillaries so that it accumulated in the liver cells, causing cholestasis and thereafter hyperbilirubinaemia Arias also demonstrated that the enzymatic conjugation of bromsulphalein with glutathione was normal, but that the liver cells were, again, inhibited in the secretion of the conjugated BSP into the bile capillaries as is seen in the clinical syndrome of chronic familial jaundice (Dubin Johnson syndrome)

As already stated, we found an increase in transaminases in 12 of our 14 patients The other liver function tests were unaffected, in particular there was no elevation of alkaline phosphatases or of serum bilirubin Case 6, a 43 year-old woman, showed a marked increase in transaminases and liver biopsy revealed cholestasis, as might be expected Goldfischer et al (9) and Schaffner et al (26), in histochemical and electron microscopic studies showed that during

administration of anabolic steroids there may be slight dilatation of the bile capillaries, there may be loss or shortening of the microvilli in these capillaries and alterations in the hepatocellular lysosomes Accordingly many findings indicate that the alkyl substituted anabolic steroids especially the 17 α alkyl substituted ones, do not inhibit the conjugation enzymes of the liver, but that in some way or other they affect certain microsomal enzyme systems operative int al in secreting conjugated substances into the bile capillaries This action is presumably always reversible According to Marquardt et al (20) methenolone, an anabolic steroid with no 17 alkyl substituent does not act upon the liver This supports the hypothesis that the hepatic action is due partially to the 17 α alkyl substitution

It has been demonstrated that methandienone causes a decrease in the urinary excretion of 17 KS and 17 OHCS and that the pituitary response in the metyrapone test was suppressed or completely blocked, while the response to ACTH remained normal The plasma cortisol level did not change during administration of methandienone but the half life of exogenous cortisol was prolonged in all the patients indicating a reduced cortisol catabolism (13, 34) In our studies, as already mentioned 11 patients showed a definite pituitary suppression in the metyrapone test, 3 of these 11 patients gave a normal response to ACTH On the other hand we were unable to demonstrate any alterations in the urinary excretion of 17 KS 17 KGS, or fractionated 17 KS There was also no definite alteration in the excretion of pituitary gonadotrophin The question is whether these alterations are due to the above mentioned hepatic action of the 17 α alkyl substituted anabolic steroids and/or to a direct suppressive action upon the pituitary hypothalamus The prolonged half life of cortisol might be taken to indicate a reduced cortisol catabolism with a consequent, transient slight elevation of plasma cortisol which by a feed back influence on the pituitary gland, causes a reduced production of ACTH

That a direct suppressive action upon the pituitary exists is supported by the fact that testosterone and its esters — which do not act upon the liver — also reduce the urinary excretion of corticoids (6) Also, the anabolic steroid methenolone lacking a 17 alkyl substituent causes as mentioned above (20), a reduced urinary excretion of 17 KS and 17 KGS, although apparently it does not act upon the liver A direct action upon the hypophysis is also supported by the reduced or completely abolished increase in the 17 KGS excretion in the metyrapone test and by the normal ACTH test

The effect of methandienone upon carbohydrate metabolism has been thoroughly studied by Landon et al (17 18) who showed that methandienone lowered the fasting blood sugar in normals as well as in diabetics and that it reduced the tolerance to oral as well as intravenous glucose The hypoglycaemic and insulin sparing effect of androgens has been demonstrated by many authors (30 31), but this effect was not demonstrable in our series On the other hand we obtained support for Landon

et al.'s findings, in that the oral glucose tolerance altered in the diabetic direction in 5 out of the 6 patients so tested. During the methandienone treatment the curve rose more rapidly and to higher values than normal. A definite explanation of this phenomenon has not yet been advanced. Landon et al. arrived at the preliminary, tentative explanation that the hepatic action of the drug — which has been described above — may inhibit or block the enzymatic uptake of glucose by the liver. It is still unknown whether a possible inhibition of glycogenolysis or an action upon the other endocrine glands is operative.

As already mentioned, the investigations of Rosenberg et al. (25) and of Federmann et al. (5) indicate that 17 α-alkyl-substituted anabolic steroids reduce the protein binding of the thyroid hormone in the serum. In accord with this conclusion, we found an increased uptake of ^{131}I-labelled triiodothyronine by the erythrocytes in 13 of our 14 patients, and determination by gel-filtration showed a fall in the protein-bound fraction of ^{131}I-labelled triiodothyronine in 10 patients. These 10 patients also showed an increased ^{131}I-labelled triiodothyronine uptake by the erythrocytes. Furthermore, the 4-hour uptake of ^{131}I by the thyroid was reduced in 11 patients and the 24-hour uptake in 13. This may be explained by the reduced binding of the hormone to plasma protein, which increases the amount of free hormone in the serum, so that the release of thyroid-stimulating hormone from the pituitary is suppressed, resulting in a reduction in the thyroid uptake of ^{131}I.

Conclusion

As stated above, we found methandienone to suppress the pituitary-adrenal response in the metyrapone test. Similarly, the protein binding of thyroid hormone in the serum was reduced and so was the ^{131}I uptake by the thyroid gland. In addition, the majority of our patients exhibited a definite increase in serum GO transaminases which, however, always returned to normal after the medication was discontinued.

Anabolic steroids and especially those which are active by mouth, i.e. the 17-alkyl-substituted testosterone derivatives have come into immensely wide therapeutic use. They are employed in a large number of situations where there is insufficient protein anabolism, especially severe infections, operations, severe burns, negative nitrogen balance during corticosteroid therapy, certain disturbances of growth in children, convalescence, geriatric situations, osteoporosis, ostogenesis imperfecta tarda, malabsorption, toxic liver damage in porphyria, progressive muscular dystrophy, thyrotoxic myopathy, Cushing's disease, metastatic breast cancer, diabetic retinopathy and nephropathy, acute and chronic uraemia, pituitary insufficiency, and hepatitis. Their use in uraemia has now been abandoned in most places and is at any rate not particularly beneficial, only in acute uraemia are they still employed (10).

Viewed in relation to this polymorphous group of diseases, the above mentioned actions of methandienone upon the pituitary, adrenals, thyroid and liver give cause for re-consideration. At any rate the use of 17-alkyl sub-

stituted anabolic steroids must be said to be contra indicated in the presence of pituitary insufficiency It is uncertain whether the esterified anabolic steroids have the same effect, a problem which calls for investigation

Summary

The effect of 1 dehydro 17 α methyl testosterone, methandienone, methyl-androstenolone (Geabol, Dianabol) on the pituitary and thyroid glands, on the adrenal cortex and the liver, and on carbohydrate metabolism was investi gated in 14 patients The majority ex hibited a suppression of the pituitary adrenal response in the metyrapone test a reduced ^{131}I uptake by the thyroid a reduced binding of the thyroid hor mone to the plasma proteins, an altera tion of the glucose tolerance curve in a diabetic direction, and a definite in-crease in the serum GO transaminase level The hepatic action is presumably always reversible In our patients the transaminases returned to normal as soon as the drug was withdrawn

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From the Department of Rheumatology (Head B Olhagen M D) the Department of Internal Medicine (Head H Lagerlof M D) Karolinska Sjukhuset the Department of Internal Medicine (Head G Biorck M D) Serafimerlasarettet, Stockholm the Department of Internal Medicine (Head H Krook M D) Centrallasarettet Vanersborg and King Gustaf V Research Institute (Head G Birke, M D) Stockholm Sweden

Precipitating Serum Antibodies to Intrinsic Factor in Pernicious Anemia

By

Ragnhild Gullberg Sten Kistner, Lars Erik Bottiger and Ulla Evaldsson

Serum antibodies to intrinsic factor (IF), or to B_{12} binding components in gastric juice, have been demonstrated in pernicious anemia (p a) patients by in vivo and in vitro techniques Extensive reviews of the different methods have recently been published e g by Doniach and Roitt, Glass, Taylor (5, 6, 7, 24) The incidence of positive serologic findings varies, the highest being 57 % in the "B_{12} transfer test" of Ardeman and Chanarin (2) An even higher percentage of positive results is obtained when p a sera are tested with parietal cells from the gastric mucosa as antigen (see 5 6 7, 24 for references)

Precipitin reactions after diffusion in gel can be used in combination with autoradiography to demonstrate antibodies to B_{12} binding antigen (11, 14 23) Preliminary results with this method concerning circulating antibodies to human and hog IF in p a patients have been reported (12) Antibodies to hog IF were demonstrated in most of the patients who had received oral therapy with hog IF, whereas antibodies to human IF were found only seldom The results suggested that different antibodies in the serum from p a patients reacted with the human and with the hog IF preparation However, only few sera were included in this investigation

In the present report sera from 78 p a patients have been studied for the occurrence of precipitating antibodies to B_{12} binding components in human and hog IF preparations Sera from 71 patients with other diseases were also investigated as well as sera from 116 persons who had undergone a health control which gave no pathological findings

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Material

Sera from 78 patients with a diagnosis of p a were studied The age and sex distribution is shown in fig 1 All patients were

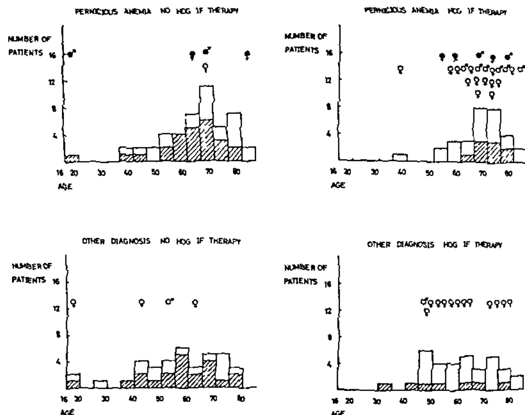


Fig 1 The age and sex distribution in the different groups of patients studied Women are represented by open columns and men by lined The symbols above the columns represent positive serologic reactions with B_{12} binders in IF preparations showing their distribution between the age groups Open symbols represent reactions to hog and closed symbols to human antigen

investigated in a hospital (Karolinska Sjukhuset or Serafimerlasarettet Stockholm or Centrallasarettet Vanersborg) The diagnosis was usually based upon the typical clinical findings of hyperchromic anemia, megaloblastic bone marrow and gastric achylia refractory to histamine Cases with any clinical suspicion of general malabsorption were excluded Schilling s urinary excretion test (UET) was performed in 51 of the patients and in 25 cases the concentration of B_{1} in serum was determined

Thirty-one of the p a patients had received oral treatment with hog IF preparation Twenty eight of these had been investigated by the UET In only two cases was the uptake of B_{12} normalized after the addition of 25 mg hog IF preparation (GEA 57 Gadex Copenhagen) in the test Forty seven patients had only received parenteral treatment with B_{1} or were untreated at the time serum was collected Twenty three in this group underwent the UET In all but one case the B_{12} absorption became normalized after the addition of hog IF The exceptional patient was a boy aged 17 who also suffered from insufficiency of the adrenal cortex He had histamine fast gastric achylia and paper electrophoresis of his gastric juice (10) showed lack of IF

Sera from 71 patients with various diseases other than p a were investigated This group included for instance cases of achylia ulcer or cancer of the stomach, hypothyroidism and thyroiditis and sideropenic anemia Thirty four of the patients had re

ceived oral treatment with preparations containing hog IF Most of the patients in this latter group were female and had a diagnosis of rheumatoid arthritis systemic lupus erythematosus or other collagen disease This selection could not be avoided due to the fact that medication containing IF is seldom given to patients without p a Such preparations have however been given to some patients with collagen disease in the present clinical material The age and sex distribution is shown in fig 1

One hundred and sixteen sera from persons who had undergone a health-check were also studied Forty were males evenly distributed between the ages of 36—45 and 56—65 respectively and 76 were females aged 36 to 70 All these persons had been found healthy after clinical and laboratory investigation They had a normal hemoglobin value and a normal sedimentation rate



Fig 2 A photograph of an autoradiogram showing the reaction between B_{12} binding antigenic component in the hog IF preparation which was put into a central well encircled by 6 wells where patients sera were added Radioactive precipitates were formed with 3 of the patients sera whereas in the other cases no reaction was seen

Methods

The patients sera were tested with a hog gastric mucosa IF preparation (WES 942 Lederle Pearl River N Y) and with human gastric juice collected after neutralization in situ (9) and concentrated by ultrafiltration Co^{58} labelled cyanocobalamin (N V Philips-Duphar Amsterdam specific activity 20—130mCi/mg) was added in an amount corresponding to the B_{12} binding capacity (12) A micro-version of the agar gel *diffusion technique (26)* was used for the antigen antibody precipitin reactions as described previously (12) Antigen and serum were tested undiluted and diluted 1 to 4 and each test run at least in duplicate The dried agar gel was left on film for about one week to visualize radioactive precipitin lines The same plates were also exposed for about one month to search for precipitates with low activity Whenever a radioactive precipitin line was demonstrated the test was repeated to check the reproducibility Fig 2 shows the result of a typical experiment

Results

Pernicious anemia patients Of 47 patients with a diagnosis of p a who had never to their knowledge taken hog IF preparation, 4 had a positive serologic precipitin reaction with B_{12}-binding components in human gastric juice Serum from one patient reacted with B_{12} binders in the hog IF preparation but in no case did serum react with both human and hog antigen The results are shown in table I and fig 1 where the age and sex distribution of the patients with positive reactions can be seen

In 5 of 31 patients with p a who had received oral treatment with hog IF a positive reaction with B_{12} binding antigen in human gastric juice was found In 19 of the cases serum reacted with B_{12} binders in hog IF preparation Two of the patients showed a reaction

TABLE I The occurrence of precipitating antibodies to B_{12} binding components in human and hog intrinsic factor (IF) preparations in the different groups of patients and controls studied

	No	Reaction to B_{12} binders in hog IF	Reaction to B_{12} binders in human IF	Reaction to B_{12} binders in hog and human IF
Pernicious anemia never treated with hog IF	47	1 (2%)	4 (9%)	0
Pernicious anemia have received hog IF	31	19 (61%)	5 (16%)	2
Other diagnosis never treated with hog IF	37	4 (11%)	0	0
Other diagnosis have received hog IF	34	13 (38%)	0	0
Health check group	116	7 (6%)	0	0

TABLE II Results of the Schilling test (UET) and test for antibodies against B_{12} binders in hog IF preparation in 31 patients with pernicious anemia who had received hog IF treatment

	No	Reaction to B_{12} binders in hog IF
UET normalized by hog IF	2	1
UET not normalized by hog IF	26	17
UET not performed	3	1

with both human and hog antigen (table I, fig 1)

Most of the patients with a serologic reaction to hog IF preparation were also refractory to hog IF in the UET (table II) In only one case did serum react with hog IF when UET showed no signs of refractoriness However, in this case the UET had been performed before the oral treatment was started, whereas the serum was collected on a later occasion Of the 26 patients resistant to hog IF in the UET, 17 had a positive serologic reaction to this preparation

Ten of the p a patients on oral therapy with hog IF were investigated on several occasions Five of these had a positive serologic reaction to hog IF preparation in the first test In four cases it was noted that a negative or weakly positive precipitin reaction became strongly positive during the course of treatment

Patients with other diagnoses None of the patients in this group had a positive serologic precipitin reaction with B_{12}-binding components in human gastric juice In 37 of the cases the history did not reveal an intake of hog IF Nevertheless, in 4 cases a positive serologic

reaction with B_{12} binders in hog IF preparation was found Two of these patients suffered from rheumatoid arthritis one had gall stones and one had gastric achylia and an irritable colon In none of these cases was a UET performed

Of 34 patients who had received preparations containing hog IF, 13 had a positive serologic reaction with B_{12}-binders in hog IF Three had a diagnosis of systemic lupus erythematosus, 4 of rheumatoid arthritis 2 of post infectious arthritis, one of Sjogren s syndrome and one of unspecified collagen disease One had a suspected multiple myeloma and one hypochylia and irritable colon Among these 13 patients a UET had been performed in 4 cases in all instances with a normal result

The distribution of the positive serologic reactions in this group of patients is demonstrated in fig 1 and table I

Health check group Of the 116 sera tested 7 reacted with B_{12} binding components in hog IF preparation (table I) Four of these were from men and 3 from women They were evenly distributed between the different age groups No serum showed a reaction with B_{12} binders in human gastric juice

Discussion

The precipitating antibodies reacting with B_{12} binding components in IF preparations which could be demonstrated in sera from p a patients as well as in sera from other individuals, seem to be of two different kinds Antibodies reacting with hog antigen were found in patients with various diseases and in healthy individuals whereas antibodies reacting with human antigen were found only in patients with p a In only two patients did serum react with both human and hog antigen Serum from 7 of the p a patients reacted only with human antigen, and serum from 18 of these patients reacted only with hog antigen This indicates that different antibodies reacted with the B_{12} binders of human and hog origin a finding in agreement with a theory previously put forward by Schwartz (21)

The B_{12}-binding property of the reacting antigen suggests that this may possibly represent IF in the preparations used The present results allow no conclusions in this respect However it has been possible to purify B_{12} binding components in the hog IF preparation by recycling gel filtration One such component which possesses IF activity when used in the UET in p a patients has been found to be immunologically identical with the B_{12} binder reacting with patients sera when tested with such sera or with experimentally produced antisera (13) Also the B_{12}-binder from the human gastric juice, which reacted with 9 of the sera from the p a patients has in the same way been found to be immunologically identical with Grasbeck s IF active binder S of human gastric juice (8) Thus it seems reasonable to believe that the precipitating antibodies characterized by their reactions with B_{12}-binding antigens were indeed IF antibodies

Antibodies to B_{12} binders in hog IF preparation were demonstrated in a low proportion of p a patients and other patients, who had not received hog IF treatment, as well as in the health check group Thus, their existence could not be correlated to the diagnosis of p a The incidence was lowest in the p a patients This could possibly be due to the fact that the information concerning previous hog IF intake was more exact in this group A temporary medication, which might have been given to other patients on earlier occasion is more likely to be forgotten and in the health check group no information at all was obtained on this matter It should be pointed out that pharmaceutical preparations containing hog IF have been rather much in use and given to patients with various conditions some years ago However, it remains possible that these antibodies existed regardless of previous hog IF medication They might be assumed to be naturally occurring heterologous antibodies, or food protein antibodies resulting from intake of pork food such as have been described in patients with various gastro intestinal disorders as well as in healthy controls (16, 22, 25)

In very few of the cases studied was a precipitin line observed after the diffusion in gel, usually they became manifest only after autoradiography Thus, little evidence was obtained for the existence of other antibodies, e g against hog protein It seems reasonable to believe that the chances for absorption—and thereby the development of antibodies — are greater with respect to IF than with respect to other large molecules When bound to B_{12} the IF is more resistant to digestion with some proteolytic enzymes (see (6) for references) Also it is possible that the absorption of hog IF benefits from specific mechanisms

The comparatively high incidence of hog IF antibodies in p a patients in whom a previous intake of hog IF was known, supports the hypothesis that the oral administration of hog IF may result in immunization The figures obtained were not as high as in the preliminary group studied (12), where 9 out of 11 p a patients on oral hog IF therapy had antibodies However, in this group all patients were on this therapy when serum was collected In the present material there were also included patients who had not been on hog IF medication for one year or more

Experimental results suggesting the existence of hog IF antibodies in p a patients on oral IF therapy have earlier been reported (17, 20) whereas other workers have not been able to confirm this (4, 15, 18) The discrepancy in the results may be explained by differences in the techniques used

Concerning the antibodies to human IF preparations, demonstrated in the present work, they were only found in p a patients Thus, they cannot be explained as naturally occurring iso antibodies or as 'spurious auto antibodies' (3) as suggested by Ramsay and Herbert (18) This last suggestion is also contradicted by the reproducibility of the reactions of the antibodies and by their similarity to experimentally produced rabbit antibodies to human IF

(12) They may be true auto antibodies, which have appeared in connection with the development of p a Unlike the auto antibodies demonstrated by other methods in p a patients the precipitating antibodies were found in only few cases This may be due to the fact that different types of antibodies are demonstrated by the different techniques (1), and that the precipitin reaction is less sensitive than other methods However, by adding anti human gamma globulin to the test system IF antibodies can be found in a higher percentage (19) This seems quite important since precipitin reactions are simple to perform and are easily accessible to analytical interpretation in gel diffusion experiments

Summary

The occurrence of precipitating antibodies to B_{12} binding components in human gastric juice and in a hog intrinsic factor (IF) preparation was investigated in sera from 78 patients with pernicious anemia (p a) 71 patients with other diseases, and 116 persons who had undergone a health-check which gave no pathological findings Antibodies to B_{12} binders in the human antigen were demonstrated in 9 of the sera from the p a patients but in none of the other sera Antibodies to B_{12} binders in the hog antigen were found in one of 47 p a patients who were untreated or treated only with parenteral B_{12} injections and in 19 of 31 p a patients who had received oral therapy with hog intrinsic factor Two of the p a sera reacted with both human and hog antigen Antibodies to B_{12} binders in the hog antigen were also found in 4 of 37 other patients in whom no intake of IF was known, in 13 of 34 patients, also without p a , who had received hog IF orally, and in 7 of the 116 healthy persons The view is discussed that the antibodies demonstrated are IF antibodies The results indicate that hog IF therapy increases the occurrence of hog IF antibodies The human IF antibodies are suggested to be autoantibodies

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From the Medical Department II Municipal Hospital Copenhagen Denmark

Retention and Distribution of B_{12} Activity, and Requirement for B_{12}, Following Parenteral Administration of Hydroxocobalamin (Vibeden®)

By

Arne P Skouby

The ideal parenteral treatment of vitamin B_{12} deficiency should secure 1) immediate abolition of the deficiency throughout the organism 2) fast restoration of natural compounds containing B_{12}, including the normal body stores 3) maintenance of an established normal condition by a few injections a year, 4) no side-effects

These demands cannot be fulfilled with preparations of cyanocobalamin (CN B_{12}) When a reasonably large amount is injected in aqueous solution most of the substance is wasted via the urine (7 12 13 16 18) When it is given in preparations containing substances producing a delayed release of the vitamin from the site of injection the organism is at the same time cut off from the amount localized in the artificial depot (8 19) Thus the fraction available for the depleted tissues at a given time will always be small

Hydroxocobalamin (OH B_{12}) might be more suitable for parenteral therapy It is as efficient as CN B_{12} in the treatment of pernicious anemia (20, 26) and is retained much better in the organism (2, 3, 6 7, 12 13 16 18 30) However, OH B_{12} binds loosely with serum and other body proteins and non bound OH B_{12} diffuses more slowly through cellophane membranes than does CN B_{12} (16) Therefore the transfer of the vitamin from the site of application to the deficient areas might be delayed, as found for CN B_{12} in preparations with delayed release

Consequently it was decided to examine the retention and distribution of OH B_{12} more closely and at the same time study the requirement for the vitamin in initial and maintenance therapy

Methods and materials

The investigation was based on determinations of the B_{12} content of serum and urine by microbiological assay using Lactobacillus leichmannii (17 31) All measurements were performed in the Department of Biochemistry Royal Dental College Copenhagen under the supervision of E Hoff Jorgensen

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Fig 2 A Serum vitamin B_{12} in normal male students after i v infusion of 200 600 and 1000 μg OH B_{12} (Vibeden®) (11 experiments) Values for serum vitamin B_{12} after i m injection of 1000 μg OH B_{12} in normal students *are given for comparison* B Calculated uptake of OH B_{12} in body cells after i v infusion of 200 600 and 1000 μg OH B_{12} in normal *controls (11 experiments) Ordinate log* scale

amounts of B_{12} activity in the tissues after administration of OH B_{12}

I v infusion of 1000 μg OH B_{12} produced changes in serum B_{12} activity which after a few hours corresponded fairly well to the changes following i m injection (fig 2 A) As the distribution from the circulation probably is independent of the site of application, the findings indicate a fast transfer of OH B_{12} from the site of i m injection into the circulation, but the lower maximum serum concentration secured a smaller initial urinary excretion following i m than following i v application

TABLE II Urinary excretion and calculated uptake in body cells of B_{12} activity after i v infusion of 1 mg OH B_{12} (Vibeden®) in normal controls and in patients with untreated vitamin B_{12} deficiency

Urinary excretion of B_{12} activity in μg	Normal controls		Patients		Difference of means	Best estimate of standard error of diff of two samples	t	p
	Mean	Standard deviation	Mean	Standard deviation				
4 hours	394	25	180	17	214	12.6	17.0	<0.001
24 hours	532	40	284	48	248	27.0	9.0	<0.001
48 hours	559	39	310	66	249	33.0	7.5	<0.001
72 hours	565	41	316	70	249	35.4	7.0	<0.001
96 hours	566	40	317	70	249	35.4	7.0	<0.001
Calculated uptake of B_{12}-activity in μg								
4 hours	265	82	487	76	222	47.4	4.7	< .01
24 hours	338	43	578	57	240	30.6	7.8	<0.001
48 hours	382	43	633	66	251	34.2	7.4	<0.001
72 hours	398	39	655	70	257	34.8	7.4	<0.001
96 hours	412	41	683	65	271	34.0	8.0	<0.001

From the results it is reasonable to assume that the uptake of OH B_{12} into body cells is faster in patients with vitamin B_{12} deficiency than in normal controls This was examined in the following experiments

Retention and distribution of OH B_{12} in patients with untreated vitamin B_{12} deficiency

I m injection of 1 mg OH B_{12} was given to seven patients The values obtained for serum B_{12} activity from 1 hour up to 10 weeks after the injection are given in fig 3 The range obtained during 3 weeks after the same treatment in eight normal individuals is indicated in the figure by vertical lines It is seen that the maximum concentration was within the same range and was obtained equally fast in patients and controls In some patients the serum concentration soon decreased to values below the range of normal controls The urinary loss within 48 hours after the injection (mean ± standard deviation) was 17 ± 8 6 per cent in untreated patients and 29 ± 9 6 per cent in the controls The difference between the values was not significant probably due to the restricted number of experiments

I v application of 1 mg OH B_{12} was tried in another group of seven untreated patients The values obtained for serum activity during 3 weeks from the infusion are given in fig 4 A and the urinary excretions after 4 48 72 and 96 hours are given in table II while the calculated amount of OH B_{12} retained in tissue cells is given in fig 4 B For comparison, average values for serum activity and cellular retention after

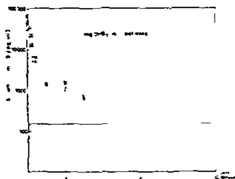


Fig 3 Serum vitamin B_{12} in patients with untreated deficiency of vitamin B_{12} after i.m injection of 1 mg OH B_{12} Vibeden[R] (7 experiments) Vertical lines indicate values obtained in normal controls Ordinate log scale

application of 1 mg to normal individuals are also given in figs 4 A and 4 B respectively The figure demonstrates that the B_{12} level of sera from patients was of the same order as or below the level of comparable sera from the controls In contrast the urinary excretion was always lower and the calculated amount of OH B_{12} retained by tissue cells higher than in the controls The differences were highly significant (table II) A couple of hours after the application 40—60 per cent and after 96 hours 60—75 per cent of the total amount was found to be bound in body cells They bound immediately about 220 μg more than cells of normal controls who had a correspondingly larger initial urinary excretion the difference increased to about 270 μg during the following 96 hours Thus the faster decrease of serum B_{12} concentration in some patients than in normal controls was due to an increased ratio of tissue binding capacity to plasma binding capacity

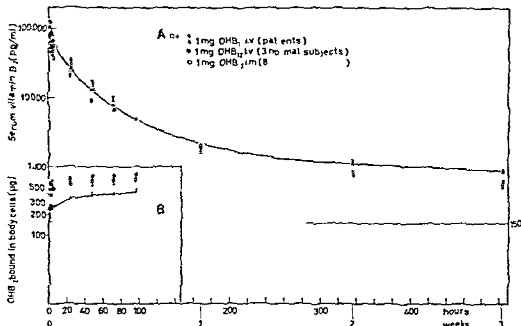


Fig 4 A. Serum vitamin B_{12} in patients with untreated deficiency of vitamin B_{12} after i v infusion of 1000 μg of OH B_{12} (Vibeden®) (7 experiments) Average values obtained in normal controls after i v and i.m application of 1000 μg OH B_{12} are given for comparison. B Calculated uptake of OH B_{12} in body cells after i v infusion of 1000 μg OH B_{12} in the patients. Values obtained in normal controls are given for comparison For details see text Ordinate log scale.

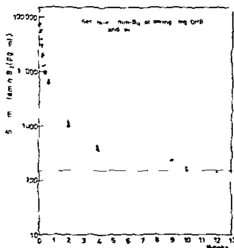


Fig 5 Serum vitamin B_{12} after i v and i m application 3 months apart, in two previously untreated patients with pernicious anemia. Ordinate log scale

The absorption of approximately 800 μg OH B_{12} from the site of injection of 1 mg in untreated patients cannot replenish depleted stores, but the rapid abolition of manifest vitamin B_{12} deficiency might produce a change in the ratio of binding affinities This possibility was examined next

Retention, distribution and utilisation of OH B_{12} following repeated doses of OH B_{12}
Repeated application of 1 mg OH B_{12} with long intervals (6—13 weeks) to seven patients produced no significant changes in the response pattern as estimated from determinations of serum activity and of urinary excretion of B_{12} activity I v infusion followed by i m injection 12—13 weeks later showed that the values for serum activity a few hours after the administration and during the following weeks were independent of the route

cho en (fig 5) Thus there was nothing to indicate an increased binding capacity of serum, nor did the extravascular capacity for binding, utilisation and storage appear to be reduced 3 months after the application of 1 mg OH B_{12} This might be due to a larger capacity for binding of OH B_{12} than for CN B_{12} Therefore the response to i m injection of 1 mg CN B_{12} 3 months after the application of OH B_{12} was examined in seven patients in four the serum B_{12} activity was near the lower limit for the normal range (150 pg/ml) The influence on the serum B_{12} activity is shown in fig 6 Mean values for eight normal controls are given for comparison The response was almost normal except that at one week the level was lower although inside the normal range In spite of the inevitable large urinary excretion (77 4 ± 11 per cent) a normal serum concentration was found for 6 weeks or more in five of six patients followed up As similar results cannot be obtained in untreated patients with vitamin B_{12} deficiency (19) the findings demonstrate that *i m injections of 1 mg OH B_{12} with 3-month intervals besides covering the daily needs also caused some replenishment of the depleted stores*

Repeated application of 1 mg OH B_{12} with short intervals (48 hours) will cause higher serum values a larger urinary excretion and therefore less total retention than obtained with larger intervals between the injections (4) However the technique may be convenient for initial treatment, for instance in hospital The effectiveness of 5 i m injections with 48 hour intervals was therefore studied by weekly measurements of

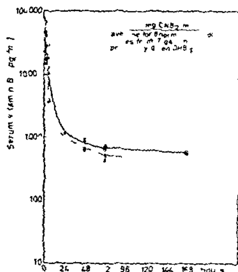


Fig 6 Serum vitamin B_{12} after i m injection of 1 mg CN B_{12} in patients given 1 mg OH B_{12} (Vibeden®) 3 months previously 7 experiments) Average values after injection of 1 mg CN B_{12} in normal controls are given for comparison Ordinate log scale

serum B_{12} concentration for several months and by examination of the influence of 1 mg OH B_{12} on the serum B_{12} activity and on urinary excretion before and 3 months after this therapy

The serum concentrations measured in six patients from 1 week up to 1 year after the last of the 5 injections are given in fig 7 During the first 8 weeks the serum level decreased to values inside the normal range and were maintained during the following months Elevated or normal values were obtained for at least 43 weeks and often for more than a year

Three subjects had another injection series 1 year after the first one Before this i m injection of 1 mg OH B_{12} caused lower values for serum activity and urinary excretion than found in normal controls I m injection of 1 mg

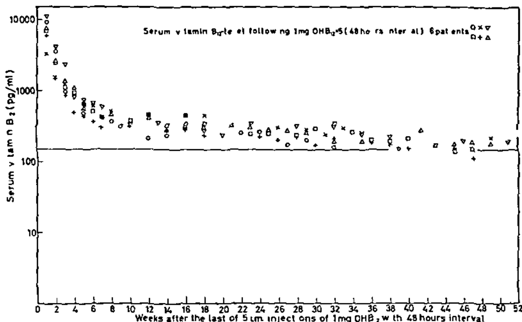


Fig 7 Serum vitamin B_{12} after 5 × 1 mg OH B_{12} with 48 hour intervals in subjects with vitamin B_{12} deficiency (6 experiments) Ordinate log scale

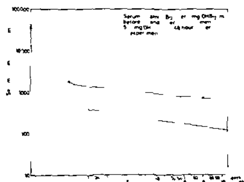


Fig 8 Serum vitamin B_{12} after 1 mg OH B_{12} i m before and 3 months after treatment with 5 × 1 mg OH B_{12} with 48 hour intervals (3 experiments) Ordinate log scale

approximately 3 months after the second series of injections gave serum concentrations within or a little above the range obtained in normal controls (fig 8) The urinary excretion within 48 hours after the injection increased from average 129 µg (65—178 µg) to average 250 µg (184—339 µg)

Thus, an important natural depot had been established in a few days by the technique used

Discussion

The study demonstrated a rapid transport of OH B_{12} from the site of application via the circulation to extravascular compartments or urine The validity of the calculated values for OH B_{12} retained in body cells hinges on the accuracy of the urine collection, the validity of the formulas used and the assumptions concerning the excretion of OH B_{12} and its distribution in body fluids Experiments where there was deficient collection of voided urine were excluded from the study, but an influence of

incomplete emptying of the bladder and contamination from one fraction to the following could not be excluded However, nothing indicated an influence of such sources of error on the results obtained for the urinary excretion of OH B_{12} within the first few hours after a load later results obtained for large volumes could hardly be influenced significantly and the total urinary excretion not at all The calculation of the amount of OH B_{12} bound in body cells was performed by means of formulas valid for normal individuals As the blood volume of patients with pernicious anemia is often reduced (5), the figures obtained might be too small However, the deviation cannot be of importance for the results As administration of approximately 1 mg OH B_{12} is followed by an enteric loss of less than 2 5—5 μg (18), it can be concluded that parenteral application of 1 mg OH B_{12} to patients with untreated vitamin B_{12} deficiency causes a retention in body cells of about 600—900 μg depending on the route of administration, the degree of depletion and the total binding capacity of the individual The patients retained immediately substantially more than did normal controls and showed during the following 96 hours a somewhat faster uptake by tissue cells These findings can hardly be due to undetected differences between extracellular compartments in patients and controls but indicate that the depleted tissues rapidly used or stored more than 200 μg OH B_{12}

The serum concentration of vitamin B_{12} is dependent on the load, the intra and extravascular binding forces and the handling of the vitamin by the kidneys In the single individual the limit for maximum retention is the highest serum concentration obtainable without urinary excretion As OH B_{12} can leave the intravascular compartment at serum concentrations not associated with a measurable urinary loss it seems likely that OH B_{12} under such conditions is passing through glomerular membranes into the tubuli but is reabsorbed, whereas CN B_{12} is treated like inulin (1, 22 29) However, the fact that OH B_{12} binds loosely with serum and other body proteins is probably of major importance for the retention in the organism during the transport via the circulation to depleted tissues for use or storage From natural depots OH B_{12} but not CN B_{12} can be isolated (11, 24) Furthermore it is demonstrable in animal experiments that OH B_{12} is retained to a larger extent in the liver than is CN B_{12} it is also more rapidly transformed to the active principle coenzyme B_{12} (14 27, 28) If similar differences exist in man these might be decisive for the better tissue retention following application of OH B_{12} than after CN B_{12}

Accordingly it appears that it is impossible clearly to delineate concepts such as depots binding capacity for cobalamins and the normal content of B_{12} activity or to exactly determine the extent of an established vitamin B_{12} deficiency However the therapeutic value of OH B_{12} is clearly demonstrated A few minutes after the application of 1 mg OH B_{12} to an untreated patient a high serum concentration is obtained and a couple of hours later a substantial amount is bound by the depleted tissues

as seen from the comparative study of patients and normal controls High to normal serum concentrations of vitamin B_1 are obtained for several weeks Following a series of five injections with intervals of 48 hours a natural depot is created covering the body needs for 10—12 months The results indicate that a repeated series a few months after the first series of injections will utilize completely the binding capacity for OH B_{12} in the organism During the regeneration period the daily demands for B_{12} activity were approximately 8 μg, there being coverage of needs and some storage with repeated i m injections of 1 mg three months apart Thus, 1 mg OH B_{12} every 3 months will always be sufficient for maintenance therapy When normal conditions are established the dose might be reduced or the interval increased As no side effects have hitherto been reported it is obvious that a preparation of pure OH B_{12} as used in the present investigation is the drug of choice for parenteral therapy of vitamin B_{12} deficiency

Summary

I m injection of 1 mg hydroxocobalamin (OH B_{12}) caused a urinary excretion within 48 hours of 29 0 $\pm$ 9 6 per cent (mean $\pm$ standard deviation) in normal controls and 17 0 $\pm$ 8 6 per cent in patients with untreated vitamin B_{12} deficiency After i v application the corresponding values were 55 9 $\pm$ 3 9 and 31 0 $\pm$ 6 6 per cent A few hours after the application the serum B_{12} concentration was independent of the route of administration in both controls and patients After i m injection the maximum serum B_{12} concentration was of the same order and obtained equally fast in controls and patients, but in some patients later values were below the range obtained in controls High to normal values for serum B_{12} activity were secured in the patients by repeated doses of 1 mg OH B_{12} 3 months apart In addition some replenishment of the depleted depots was obtained

I v infusion of 200, 600 and 1000 μg OH B_{12} caused serum B_{12} concentrations and urinary excretions which increased with the load in individuals with normal body stores of vitamin B_{12} Calculations showed that the amount taken up by body cells increased but not in proportion to the load After 96 hours — more than 24 hours after cessation of the urainary excretion of B_{12} activity — it was 62, 53 and 41 per cent respectively The amount retained in body cells of untreated patients after i v infusion of 1 mg OH B_1 was higher than in persons with normal stores The discrepancy appeared soon after application, which indicates that depleted tissues immediately retained more than 200 μg of the load After a few hours 40—60 per cent and after 96 hours 60—75 per cent of the dose was retained in body cells

5 i m injections of 1 mg OH B_{12} with 48 hour intervals gave high to normal serum B_{12} activity for at least 43 weeks and usually for approximately one year Three months after a series the response of serum B_{12} activity and urinary excretion to application of 1 mg OH B_{12} was inside the range for controls

with normal body stores of vitamin B_{12} build up by physiological mechanisms for absorption and storage

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From the Departments of Skin and Venereal Diseases (Head G Asboe Hansen M D) and Obstetrics and Gynaecology (Head Dyre Trolle M D) Rigshospital University of Copenhagen Copenhagen Denmark

Urticaria Pigmentosa Associated with Thyroid and Ovarian Goiter

By

FINN RASMUSSEN

Urticaria pigmentosa is the cutaneous manifestation of mastocytosis The disease has been known for about a century (11), but its universal character with accumulation of mast cells in bones bone marrow, liver lungs spleen and other organs containing mesenchymal tissue has been realized only within the last 10—15 years (3, 13) In spite of its rather infrequent occurrence, many cases have been reported in the literature In 1960 Sagher and Even-Paz gave a total figure of 500—600 cases

In this paper a case of mastocytosis in a young woman with ovarian goiter is reported with the aim of finding what connection, if any, exists between the two diseases

It is a well known fact that mast cells are influenced by hormones Corticotropin and adrenal corticosteroids cause the mast cells to become small and irregular with vacuolized cytoplasm and conglomerated granules (2 21) Thyrotropin produces an increased number of mast cells of considerable size and abundantly granulated whereas mast cells are seen to be few small and degranulated after administration of thyroxine (1 4, 17)

No relation is known to exist between mastocytosis and diseases of the thyroid gland Thyroxine intensifies the effect of adrenaline, probably by inhibiting its breakdown (6) Due to the fact that one of the functions of the mast cells is production of histamine it seems likely that thyrotoxicosis might foster mastocytosis as a compensatory reaction of tissue to the increased influence of adrenaline On the other hand there is some reason to suppose that the enormous number of mast cells in mastocytosis with an accompanying increased production of histamine might cause a compensatory thyroxine over production

Case report

The patient admitted to the Department of Dermatology and Venereology of the Rigshospital was an eighteen year old student

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Fig 1 Urticaria pigmentosa papules on the front of both thighs.

During the past two years she had noted some brownish, itching spots in her skin, starting on the extremities later on appearing over the trunk, and at the admission on her face too Scratching gave rise to urticarial streaks. Apart from slightly irregular menstruations she had been in good health

Five years earlier her forty three year old mother had been operated on for colloid goiter because of mild thyrotoxic symptoms. Before the operation her metabolic rate was 110—116 per cent. PBI was 9 4—6 8 μg 100 ml. One sister died at the age of twenty after an accident. She was in good health. No post mortem examination was made A younger sister was fourteen years old and was put under observation in a children s hospital under the diagnosis non toxic diffuse goiter Her latest metabolic rate was 110 per cent PBI was 5 1 μg/100 ml No cases of urticaria pigmentosa were reported in the family

Examination of the patient revealed a typical urticaria pigmentosa of the maculopapular type (fig 1) Urticarial dermographism could be demonstrated both in the pigmented spots and in the normal skin In addition she had a slightly diffuse cervical goiter and gynaecological examination showed a tennis-ball-sized tumor to the left of the uterus

Because of the last mentioned finding the patient was admitted to the gynaecological department for operation At this operation her left ovary was removed It was found transformed into a multicystic tumor 8 × 6 × 5 cm in size (fig 2) The right ovary was slightly enlarged with two small cysts

Histopathology

A skin biopsy sample fixed in a 4 per cent aqueous solution of lead subacetate showed the epidermis to be of normal thickness with slight acanthosis. There was a rather heavy melanin pigmentation of the basal layer In the superficial third of the connective tissue there was a considerable accumulation of mast cells Some were located around the vessels as sheaths whereas others were single In this area the ground substance was found to be strongly metachromatic The



A



B

Fig 2 The multicystic tumor in the left ovary A In toto B Cut through.



Fig 3 Photomicrograph of skin biopsy showing melanin pigmentation of the basal epidermic layers and accumulation of mast cells in the connective tissue Staining toluidine blue 0 5 % aqueous solution Magnification × approx 300

deep layers also revealed a pathologically increased number of mast cells but the frequency decreased with increasing depth On the whole the corium was thickened and fibrous Sweat glands smooth muscles one hair follicle and one sebaceous gland showed no abnormality (fig 3) Micr diagn urticaria pigmentosa (sign G Asboe Hansen)

Skin histamine was determined by spectrofluorometry (19 20) In the urticarial lesions it was 57 3 μg per mg dried defatted tissue which is an elevated value whereas the content of histamine in the normal skin was 31 9 μg per mg dried defatted tissue (normal)

Microscopy of various sections of the extirpated ovary showed smooth walled cysts as well as small areas consisting of normal ovarian stroma In the sections



Fig 4 Photomicrograph of the left ovary showing typical thyroid tissue Staining hematoxylin and eosin Magnification approx 150

widespread areas with thyroid tissue were found which were composed of closely packed rather varying follicles lined by a flat cubic epithelium and filled up by colloid There were no signs of inflammation nor necrosis or malignant alterations and there were no signs of teratoid formations (fig 4) Micr diagn struma ovarii (sign Wanstrup)

The cysts contained a total of 95 ml of yellowish serous fluid of pH 7 6 After adjustment of the pH to 5 0 acid mucopolysaccharides were precipitated with an ad mixture of 1/10 volume of 5 per cent cetavlon This produced a precipitate of 192 mg net weight after drying Paper electrophoresis showed bands intensely stained with Alcian blue corresponding to hyaluronic acid and chondroitin sulphate an intermediary band moving rather slower than chondroitin sulphate and furthermore a strongly stained non migrating band which probably represented mucopolysaccharides bound to proteins By means of the carbazole method (8) the uronic acid content in the precipitate was assessed as 10 5 mg/100 mg dried tissue

Laboratory findings

Sedimentation rate 6 mm/hour Hemoglobin 12 4 g/100 ml Leucocytes 5 900/μl Differential count neutrophils 60 % lymphocytes 35 % monocytes 5 % Erythrocytes normal Direct counting of the eosinophils 181/μL Thrombocytes 248 000/

µl. Serum creatinine 0.7 mg/100 ml. Serum cholesterol 198 mg/100 ml. Paper electrophoretic determination of serum proteins Total 8.24 alb. 5.18 alpha-1 glob 0.39 alpha-2 glob. 0.64 beta-glob 0.78 gamma glob. 1.25 Coagulation pattern Slightly increased prothrombin-proconvertin (Owren's p-p 130—160 %), other values normal. PBI 5.3 µg/100 ml. Basal metabolic rate 97—93 %. Blood pressure 120/80 mm Hg.

Urine 5-hydroxyindoleacetic acid 0. 17-ketosteroids 5.04 mg/24 hours (normal). No alb. or sugar. Quantitative determination of acid mucopolysaccharides 3.2 mg glucuronic acid (normal) and 1.5 mg hexosamine per 24 hours (normal).

X-ray examination of chest and skeleton no abnormality.

Eyes slight strabismus convergens sin., otherwise no abnormality.

Six months after the operation the patient was readmitted to the dermatological department for check up. She was feeling well, and her menstruations were normal. Her urticaria pigmentosa was essentially unchanged except for a few new elements. Gynecologic examination revealed the right ovary to be slightly enlarged. The laboratory findings were normal.

Discussion

Ovarian goiter, or struma ovarii, is defined as a teratoid tumor entirely or mainly consisting of thyroid tissue (7, 9, 15, 18).

Usually the disease is diagnosed in middle-aged women, rarely in patients below the age of 25 years. In most cases the ovarian tumor contains cysts filled with colloid and lined by cubic or flat epithelium (12, 16). As is the case in the thyroid gland, these cysts are considered to arise by degeneration or fusion of big follicles (16). Usually the disease is incidentally observed at a routine gynecologic examination. In the case reported, the patient had irregular menstruations. Besides the menstrual disturbances the following symptoms may be encountered — Compression of the surrounding organs, ascites, and, in 5 to 6 per cent, thyrotoxicosis (10). This patient was euthyroid. She had a slightly diffuse cervical goiter, a feature which, according to Smith (15), is evident in 16.3 per cent of ovarian goiter cases.

As the patient was not thyrotoxic, her mastocytosis could hardly have been secondary to an overproduction of thyroxine, caused by the ovarian tumor. The converse has never been supported by clinical observation.

Consequently, so far we can only presume a connection between the thyroid gland and mastocytosis. This paper is published in the hope that it might be one of the elements needed to construct the complete picture, the complete understanding of the pathogenesis of a mysterious disease.

Summary

A case of urticaria pigmentosa with thyroid and ovarian goiter in an eighteen-year old woman is reported. The diseases and the possible mutual relations are discussed.

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TABLE I The results of arterial blood-gas analysis on admission and on discharge about 12 weeks later

	Arterial blood gases (breathing air)			
	HbO_2 (%)	Total CO_2 (mEq/l)	pH	pCO_2 (mm Hg)
On admission	50 9	47 6	7 27	101 0
On discharge (12 weeks later)	91 8	30 5	7 41	47 6

with use of the Beckman spectrophotometer after Refsum and Sveinsson s modification (10) Arterial pH was measured with a Radiometer pH meter Total CO_2 and pCO_2 were calculated according to Siggaard Andersen et al by the Astrup method (14)

In table I, values for arterial hemoglobin oxygen saturation, pH and total CO on admission are given In the same table the results of the examination of blood gas analysis at the discharge 12 weeks later also are given

As seen from the figures, serious hypoxemia with arterial hemoglobin oxygen saturation 50 9 % hypercapnia with carbondioxide tension 101 0 mm Hg and respiratory acidosis with pH 7 27 were present on the admission, and artificial ventilation was considered indicated

A tracheotomy was performed and the patient was connected to a Bennett intermittent positive pressure respirator with a rate about 20—22 per minute and pressure 25—28 cm water

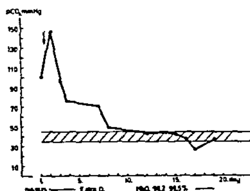


Fig 1 Changes in pCO_2-values during the first 20 days with artificial ventilation Arrow marks the leakage

Fig 1 shows the changes in pCO_2 during the artificial ventilation Because of a tracheal leakage during the first 24 hours with additional supply of oxygen pCO_2 increased to 145 mm Hg After correction of the position of the tracheal cannula the course of the artificial ventilation was uncomplicated Arterial hemoglobin oxygen saturation immediately became normal due to oxygen enrichment Normal values for pCO_2 were reached about 10 days after admission

The patient gradually recovered, but needed intermittent therapy with artificial ventilation for another 7 weeks He was discharged 12 weeks after admission Values for the arterial blood gas parameters at this time are given in table I and show that there still persisted a slight hypoxemia and hypercapnia

On discharge the weight was reduced to 98 5 kg X ray of the chest showed a normal heart size but the pulmonary vessels were somewhat prominent His vital capacity was then 2 145 ml (BTPS), $\frac{FEV_{1.0}}{VK}$ 100 = 65 3 % and maximal voluntary ventilation 55 5 l/min (BTPS)

Discussion

The case reported showed on admission a severe respiratory failure with a low arterial hemoglobin oxygen saturation (50 9 %) a low arterial pH (7 27) and an increased arterial carbon — dioxide tension, *(101 0 mm Hg)*

During the stay in hospital, examination of the lungs revealed no actual pulmonary disease The primary cause of his ventilatory insufficiency was therefore his extreme adipositas ('Pickwickian syndrome')

Usually, the ventilatory dysfunction in these patients is of relatively minor importance, and a reduction in weight has induced improvement (7, 11, 15) But as shown in our case, the respiratory insufficiency can be serious and cause severe disturbances in the arterial blood gases

When dealing with aspects of the development of respiratory failure in patients with extreme obesity, several authors (3, 8 9) have *concluded that* the adipositas represents a mechanical burden on the respiratory system, leading to an impaired bellow function of the chest with alveolar hypoventilation

Other authors (2 5 13), have stated that in many patients with Pickwickian syndrome , a characteristic finding has been a pronounced reduction in the arterial oxygen tension, with normal or only moderately elevated values for carbon dioxide tension This could indicate that the abnormality was due to a disturbed ventilation perfusion relationship

In our case, the results of arterial blood gas analysis on admission revealed hypoxemia with a corresponding hypercapnia (Refsum (12)) When additional oxygen was administered, the arterial hemoglobin oxygen saturation rose to normal values within few minutes, which shows that an alveolar hypoventilation was the main reason for the respiratory failure and that possible disturbances in ventilation perfusion ratio have been of minor importance After about eight weeks of artificial ventilation and on low caloric diet the patient was able to maintain normal blood gases, even during spontaneous ventilation

Summary

A case with extreme obesity, dyspnea and somnolence ('Pickwickian syndrome) is reported The obesity induced alveolar hypoventilation with resulting severe hypoxemia, hypercapnia and respiratory acidosis

After eight weeks of artificial ventilation and low caloric diet the blood gases became normal, even during spontaneous ventilation

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From the First Medical Service and Department of Clinical Chemistry Sahlgrenska sjukhuset University of Goteborg Göteborg Sweden

The Composition of Human Subcutaneous Adipose Tissue in Obesity

By

Per Björntorp Bertil Hood, Alf Martinsson and Bengt Persson

The increase of adipose tissue in obesity can be due to a hypertrophy of the preexisting cells in the fat depots i e an increase of the amount of fat in each cell or to hyperplasia an increase in the number of fat cells In addition the cytoplasmic mass could vary, as could also the part of the adipose tissue that consists of extracellular space These problems have been approached through the determination of different spaces in vivo in human obesity by Ljunggren et al (8), who demonstrated that both hypertrophy and hyperplasia were present The same results have been obtained by morphological techniques (2 11)

The concentrations of different constituents in adipose tissue in human obesity have been investigated previously (5 10 13 14) However, only recently in a few cases of obesity have data including cell number, cytoplasmic mass and triglyceride concentration been reported (7) Such observations are necessary for a more complete understanding of obesity but also in studies of human adipose tissue in vitro it is important to take into consideration the cellularity of the tissue relating different activities to units of references as has been done for the mouse 6 and rat (16) In the present investigation determinations have been made of different substances in adipose tissue as well as of adipose tissue cell size and cell number, so as to investigate to what degree hypertrophy or hyperplasia is present in obesity and how the cytoplasmic mass of adipose tissue varies with respect to body weight

Material

Obese group Six women 17—68 years of age mean age 42 years had no glucosuria fasting blood sugar below 90 mg per 100 ml a glucose disappearance rate (1) corresponding to a k value of 1.25 ± 0.14 (mean $\pm$ standard deviation) and were all more than 25 % above ideal weight (9) In these cases there was a history of overeating with a weight increase over a period of years preceding the study Two of the women

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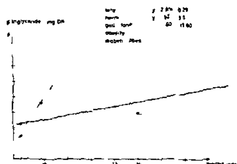


Fig 1 Correlation between g triglyceride/mg DNA of human subcutaneous adipose tissue and weight index for different clinical groups Total material r 0 57 p < 0 025 Hernia r 0 72 p < 0 05 Gall stone r 0 99 p < 0 001

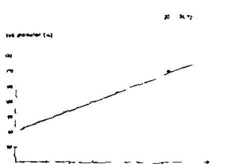


Fig 2 Correlation between fat-cell diameter in subcutaneous adipose tissue and weight index for different clinical groups symbols as in fig 1 Total material r 0 84 p < 0 0001

gall stone groups There was no significant difference between the two obese groups but they had a higher triglyceride content per unit DNA than the hernia patients ($p < 0.025$ for the obesity group $p < 0.05$ for the diabetic obesity group) although not higher than the patients with gall stones The content of phospholipid phosphorus per unit of DNA was virtually identical in these groups Fat cell size was significantly higher ($p < 0.001$) for both obese groups than for the hernia and gall-stone patients while the patients with cholelithiasis were not significantly different from the hernia patients Both obese groups had a significantly lower number of fat cells per mm^3 than the two other groups ($p < 0.001$)

In fig 1 triglyceride/DNA is plotted against weight index calculated as actual weight/ideal weight (9) For the whole material there was a significant correlation ($p < 0.025$), and among the subgroups this was true also for the patients with hernias ($p < 0.05$) Also in the small material of patients with gall stones there was a sinificant correlation ($p < 0.001$) Fig 2 shows the relationship between cell diameter and weight index Here too a significant correlation was found for the whole material ($p < 0.0001$)

Discussion

Morphological as well as chemical measurements have been used for cell size determinations in this investigation in a manner described previously (3) The fact that essentially the same results were obtained whether morphological or chemical data were used as a measure of cell size supports the earlier suggestion (3) that they are equally adequate for this measurement

It was shown that the content of triglyceride per unit DNA was greater in the obese groups than in the group with hernias Mean fat-cell size was greater in the obese groups than in the hernia group and gall stone group This implies that in the obese groups there was a hypertrophy of the cells,

with more triglyceride per cell than in the group with hernias Using the cube roots (2) of the cell number values given in table I, one can calculate the number of fat cells per mm distance along a length of adipose tissue Comparison of the figures thus obtained reveals that the non obese groups have at mcst 40 % more cells per mm adipose tissue than the obese

If hypertrophy of fat cells were the only factor contributing to increase of adipose tissue in obesity, the same total number of fat cells would have been found in the obese and non obese groups

From the figures for cell number per mm it would follow that there is an increase of adipose tissue thickness in the obese group of less than 40 %, if the increase of adipose tissue volume is assumed to be equal in all dimensions Skin fold measurements performed in some of the cases indicate a difference in adipose tissue thickness of at least 300 % between the obese and non obese groups Thus, with the assumption mentioned above a considerable increase of adipose tissue cell number would seem to have been present It is doubtful, however, whether the assumption is valid that adipose tissue increases equally in all dimensions, since body surface area probably increases relatively less than the thickness of adipose tissue The question whether hypertrophy or hyperplasia dominates in obesity thus has to be left open It seems clear, however, that hypertrophy of adipose tissue cells is almost constantly present, although to a varying degree as judged from the scatter of fat cell diameters in fig 2

In all the groups investigated there was almost the same concentration of phospholipid phophorus per unit of DNA Under the premises discussed previously (3) this would mean that the cytoplasmic mass, or at least the phospholipid containing membranous structures of the cells, is present to the same degree in the fat cell whatever the content of triglyceride or the body weight

Summary

Triglyceride, phospholipid phosphorus, and deoxyribonucleic acid levels as well as fat cell size and number of fat cells per mm³ were determined in human adipose tissue taken from patients operated upon for abdominal hernia or for gall stone disease without biliary obstruction and from patients with obesity both with and without diabetes mellitus With increasing body weight there was an increase in the amount of triglyceride per unit of deoxyribonucleic acid as well as in the mean diameter of the fat cells The concentration of phospholipid phosphorus per unit of deoxyribonucleic acid was almost identical in the different groups which indicates that cytoplasmic mass per adipose tissue cell is not increased in obesity It was concluded that, in obesity in the human hyper trophy of fat cells, viz increase of fat content, is present to a varying degree

Acknowledgements

This work was supported by grants T 304 W 253 and Y 495 from the Swedish Medical Research Council

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From the First Medical Service and Department of Clinical Chemistry University of Goteborg Goteborg Sweden

The Sucrose Space of Human Subcutaneous Adipose Tissue in Obesity

By

PER BJORNTORP, BERTIL HOOD and ALF MARTINSSON

The participation of adipose tissue water in the weight increase of obesity is a matter of discussion Studies of body composition on living persons indicate a considerable increase of total extracellular water in obesity, especially in certain clinical entities of this disorder (11)

Morse and Soeldner (12) found no differences in either total water content or sodium space in human adipose tissue from obese and non obese persons in studies performed in vitro Bozenraad (5) Pawan and Clode (13) and Thomas (14) have however found that obese persons have lower amounts of water in adipose tissue Kahlenberg and Kalant (8) investigated the sorbitol space of omental adipose tissue in diabetic and non diabetic patients and found no differences in the extracellular space

When working with human subcutaneous adipose tissue in vitro the period of time for equilibration of the tissue with the incubation medium was determined for each sample in order to check the diffusion during the incubations (4) These data were considered to warrant separate publication since they seem not only to contribute to the question of tissue permeation but also to give values for sucrose (extracellular) space in human adipose tissue at different body weights

Submitted for publication January 13 1966

Material

The clinical material consisted of 13 obese patients weighing more than 20 % over ideal weight (10) which were compared with 10 control patients A detailed description of the material has been given elsewhere (4) The patients were classified into the following four groups

The diabetic obese group consisted of six patients with constant glucosuria but without need for insulin treatment

The obese group consisted of seven patients with no signs of clinical diabetes mellitus

The hernia group and the gall stone group consisted of six and four patients respectively These 10 control patients were all within ± 13 % of ideal weight (10)

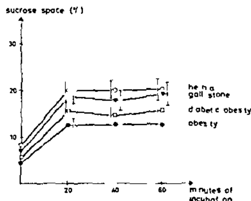


Fig 1 Sucrose space after different periods of incubation of human subcutaneous adipose tissue in vitro from different clinical groups Means ± SEM

TABLE I Sucrose space of human subcutaneous adipose tissue from different clinical groups Means±SEM

	Sucrose space[1] (%)
Hernia	20 2±1 2
Gall stone	18 0±1 5
Diabetic obesity	15 2±1 1
Obesity	12 8±1 8

[1] Means of measurements at 20 40 and 60 minutes (cf fig 1)

Methods

Biopsy of adipose tissue was performed as described previously (2) The adipose tissue was cut into pieces each weighing 25—40 mg and kept at room temperature in 4 % albumin (Bovine albumin Armour fraction V Batch Hb 1371) in Krebs Ringer bicarbonate buffer of pH 7 4 containing glucose at a concentration of 1 8 mg per ml They were carefully blotted with a filter paper and weighed on a torsion balance Then thirty minutes after the surgical removal four pieces were incubated in 2 ml of the albumin containing buffer with sucrose U ^{14}C (The Radiochemical Centre Amersham, CFB4) corresponding to about 2×10^6 counts/min Directly after addition of the piece and after 20 40 and 60 minutes respectively, one piece was removed from the incubation medium rinsed during less than five seconds in about 4 ml of the incubation medium without labelled sucrose and then transferred to 1 ml water which was brought to boiling

To 0 1 ml of this extract 0 5 ml Hyamine 10 X (Packard) and 0 5 ml absolute ethanol was added in that order and then 10 ml of 0 4 % PPO (Packard) and 0 01 % dimethyl POPOP (Packard) in toluene Counting was performed in a Packard Tri Carb liquid scintillation counter Quenching was corrected for by internal standard

Since homogenization of the tissue after boiling did not increase the radioactivity of the extract the above mentioned procedure was considered adequate

The radioactivity in 10 μl of the incubation medium was also counted in the way mentioned The radioactivity in the tissue was assumed to be present in the tissue water in the same concentration as in the incubation medium The volume of water thus obtained from determination of the radioactivity was expressed as per cent of tissue wet weight and was after equilibration called the sucrose space

Cell size and deoxyribonucleic acid (DNA) were determined as described earlier (2)

Results

Fig 1 gives the measured sucrose space at different times after incubation for the groups investigated It seems that in all groups irrespective of the size of the sucrose space, a steady level was reached within 20 minutes

The sucrose spaces found are listed in table I The hernia group had a significantly higher space than the obese groups ($p < 0\ 01$ for the obese group and $p < 0\ 05$ for the diabetic obese

group) and the gall stone group had a higher space than the non diabetic obese group ($p < 0.05$)

In fig 2 the relation is shown between sucrose space and body weight given as the weight index actual weight/ideal weight (10) No rectilinear correlation appeared for the whole material, but as judged visually, there seemed to be a levelling off of the rapid initial fall of sucrose space at weight indices above 1 This result further prompted plotting of sucrose space against cell size (fig 3) and also a chemical expression of this (2), viz the ratio of triglycerides to DNA (fig 4) The results seemed to be essentially the same as when weight index was used as abscissa (fig 2), viz a levelling off of sucrose space after an early rapid fall at a cell size of about 100 μ (fig 3) or otherwise expressed at about 3 g triglycerides per mg DNA (fig 4)

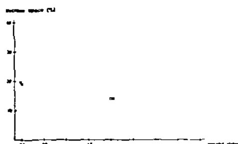


Fig 2 Correlation between weight index and sucrose space in human subcutaneous adipose tissue in vitro (symbols as in fig 1

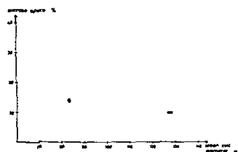


Fig 3 Correlation between sucrose space and mean cell diameter in human subcutaneous adipose tissue in vitro (symbols as in fig 1

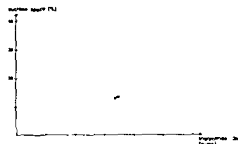


Fig 4 Correlation between sucrose space and grams triglyceride per mg DNA in human subcutaneous adipose tissue in vitro (symbols as in fig 1)

Discussion

The sucrose space of adipose tissue in vitro from patients with normal weight was thus found to be higher than that of patients with obesity

Sucrose is a substance which distributes itself mainly in the extracellular space of the tissue, since it seems unable to pass the cellular membrane as such (7) It has also been demonstrated that sucrose is not converted into carbon dioxide or lipids in human adipose tissue (9) The sucrose space accordingly will approximate to the extracellular space of the tissue

Larger pieces of adipose tissue than the ones utilized in the present work seem to increase their total water content during incubation (9) This did not seem to be the case in the tissues used in the present work since the sucrose space was unchanged at 20 40 and 60 minutes of incubation It is possible that an increase

of water content may have occurred when the tissue was kept in buffer solution before incubation for sucrose space measurements, or that water content may increase only in adipose tissue pieces of higher weight or, possibly, that other water spaces, not measured, may increase during incubation

It has been shown earlier (3) that weight increase correlates with an increase of triglyceride content per fat cell Taken together with the present findings, this seems to indicate that when adipose tissue mass increases, not only an absolute but also a relative increase of triglycerides occurs since extracellular space, and thus probably total water (9), shows a relative decrease

Equilibrium between tissue and the labelled sucrose was attained already within 20 minutes of incubation with all tissue samples studied Thus, even with the comparatively thick pieces of tissue, equilibrium was rapidly obtained It is possible that even larger pieces of tissue equilibrate rapidly enough with the incubation medium to allow measurements of different metabolic activities These problems are currently under investigation (9)

There did not seem to be a rectilinear correlation for the whole investigated material between different body weights and the sucrose space The results were essentially the same whether the cell size against which the sucrose space was plotted was measured by morphological techniques or chemically, viz triglyceride amount per unit DNA in adipose tissue This phenomenon might not be considered as definitely demonstrated because of the limited material, especially in the region of subnormal weight, but it is of interest to note the agreement between this finding and the predicted extracellular space of adipose tissue from the human as deduced by morphological mathematical techniques by Bjurulf (1)

Even though the data presented seem to be of interest mainly in that they enable comparisons to be made between different clinical groups, they might also be a guide to absolute body water spaces in vivo It thus seems probable that a substantial amount of extracellular water is present in adipose tissue in obesity In cases of obesity with a small mean diameter of the fat cells and thus a comparatively large extracellular space (cf fig 3), this volume of water might at least partly offer an explanation for the water retention in so-called pathological obesity (11)

Summary

Sucrose space was determined in vitro in human subcutaneous adipose tissue from patients of different body weights The obese patients had a smaller sucrose space than controls There were higher values for sucrose space at lower body weights, while at higher body weights this space seemed to level off

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Alteration of time *The International Conference on Liver Regeneration* will be held in Montecatini-Terme, Italy, on October *29—30*, 1966, under the Chairmanship of Prof Mariano Messini, Director of the Postgraduate School for Liver Diseases, University of Rome

Organizing Committee Milan, Via Modica 6, Italy

Acta Medica Scandinavica Vol 180, fasc 2, 1966

From the Department of Internal Medicine (Head N Tornblom) and the Department of Clinical Physiology (Head H Linderholm) University of Umeå Umeå Sweden

Double Blind Trial with Prenylamine (Segontin) in Coronary Insufficiency

By

K A JACOBSSON GUNTER KOCH MAJ LEVANDER LINDGREN and GUNNAR MICHAELSSON

The treatment of coronary insufficiency with drug is unsatisfactory Among agents considered to produce coronary vasodilatation Segontin N [3 phenyl propyl (2)] 1 1 diphenyl propyl (3) amine (prenylamine) has met growing interest

In experimental animals Segontin has been shown markedly to increase the resting coronary blood flow without significantly increasing myocardial oxygen consumption (7 16 19) Dilatation of the coronary arteries has been demonstrated by coronary cinearteriography in cats (8) Segontin has local anaesthetic but no analgesic properties It also has a weak sympatholytic and in high doses a sedative effect in chickens and rats (19) Apparently, Segontin interferes with catecholamine metabolism It has been shown markedly to reduce the noradrenaline and to a lesser degree the serotonin content of the myocardium (27 28)

Haemodynamic investigations in man have been carried out by Abrahamson in 1963 (1) In patients with mitral stenosis he found a slight decrease in the systolic pressure, the pulmonary wedge pressure and the cardiac output Segontin induces a slight decrease in pulse rate in healthy men but there is no well defined effect on blood pressure (21) Using coronary angiography no clear Segontin induced vasodilatation could be demonstrated in arteriosclerotic heart disease (18)

The toxicity in the animal is low both in single doses and during prolonged administration (19) High doses have a slight sedative effect in man and vertigo has been reported in some cases Rare instances of skin erythema headache and gastro intestinal symptoms have been reported but other serious side effects e g on haemopoesis and liver and kidney function have not been observed (2 3 5 7 9 10 12 17 20 22 24 25 31 33

Openly conducted *clinical trials* (5 6 10 11 12 17 24 25 31 33) and double blind trials (2 3 9 15 20 22 23)

Submitted for publication November 12 1965

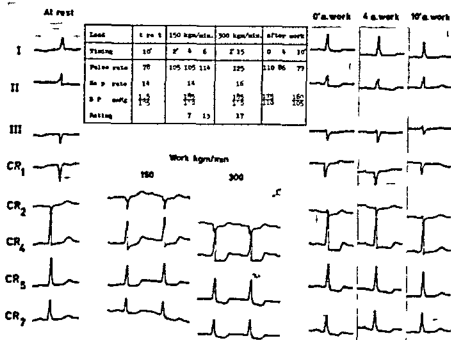


Fig 1 ECG reaction typical of coronary insufficiency during work test (patient E H 52 year-old)

using a dose of 30—180 mg/day have shown a decrease in angina pectoris in the majority of cases Divergent results have been obtained with objective methods such as ECG examination and the Master s test (3, 5, 9, 11, 12 15 20 31)

The purpose of the present investigation was to assess the results of treatment of coronary insufficiency with Segontin regarding subjective symptoms as well as objective changes occurring during a calibrated exercise tolerance test on a bicycle ergometer

Material

Male patients with a clear history of angina pectoris of a coronary type and an ECG *reaction* typical of coronary insufficiency were selected

The ECG reaction considered typical of coronary insufficiency in this study is shown in fig 1 and defined as follows

Occurrence or exaggeration of ST depression during work giving a horizontal or downward sloping tracing with flattening of the T wave and an eventual initial negative deflection These changes increase fairly proportionally to the work load the ST changes reaching their maximum at the *highest work load or immediately* after the exercise and returning gradually to the pre work ECG pattern The T wave changes occasionally may be most pronounced 4 minutes after work These criteria correspond mainly to those of other investigators (26)

The final criteria for selection were a normal or only slight increase in heart volume absence of symptoms of decompensation arterial hypertension of a degree demanding treatment signs of recent infarction or of other complicating disease In all cases the clinical course of the disease had been

stable for at least 3 months and no digitalis preparation had been given

10 co-operative patients who fulfilled all these criteria were selected having the following characteristics

Age range 43—63 years mean 55 years

Duration of angina pectoris range 6 months—7 years mean 3 years 2 patients had a previous myocardial infarction

Concurrent diseases One of the patients had a senile diabetes treated with tolbutamide Another patient had been on anticoagulant therapy (Sintroma) for 3 months before the trial started with a satisfactory and constant prothrombin index

Total serum cholesterol was determined in 9 cases and found to be markedly elevated in all (315—595 mg/100 ml serum)

Blood pressure range at rest was 135—170/80—105 mm Hg

ECG at rest was normal in 3 cases the others having ST T segment depression During the exercise test all of the patients had an ECG reaction typical of coronary insufficiency

Heart volume range 325—560 mean 430 ml/m² body surface area

No drugs except nitroglycerine and eventually sedatives at night were allowed besides the trial medicine Conditions of working activity were constant throughout the trial period

Methods

Exercise test

The patients were exercised in the sitting position on an electrodynamically braked bicycle ergometer (13) starting at a load of 150 200 or 300 kpm/min This was increased by steps every sixth minute (34 30) by a further 150 200 or 300 kpm/min until the patient considered himself unable to continue because of precordial pain Maximal working capacity (W_{max}) was calculated as the greatest load at which the patient worked for 6 minutes with a proportional increment if he completed part of the period of the next higher load (32)

The ECG was recorded at rest in the supine position during exercise on the bicycle ergometer in the sitting position and again in the supine position immediately and 4 and 10 minutes after exercise using standard and precordial leads (28 14) At work the ECG was recorded after 2, 4 and 6 minutes at each load During the fourth minute at each load and usually immediately before exercise was interrupted the blood pressure was measured according to Riva Rocci At the same time the respiratory rate and the patients subjective judgement of the working intensity according to a rating scale (4) were recorded along with a description of the type of pain and its time of onset and disappearance

After the first orientating work test loads were selected to allow the patient to exercise at least throughout two but preferably three working loads before the test had to be discontinued Two series of double blind trials of the preparations were conducted one with intravenous injection and the other with oral administration

Injection trial

The different preparations called Ugontin A D and E (which were revealed to contain 220 mg Oxyethyltheophylline 10 mg Segontin and saline respectively after opening the code) were given on three consecutive days after the control test during the patient s stay in the hospital The test was always done at the same time of day (3—4 p m.) The intravenous injection was given during 10 minutes and the exercise tolerance test started 5 minutes after completion of the injection

The exercise tests were analysed with regard to

1 Time lag i e time in minutes until onset of precordial pain

2 Duration of precordial pain after the end of exercise

3 Physical working capacity calculated as W_{max}

4 Maximum heart rate during the exercise test

5 Patients rating of the working intensity

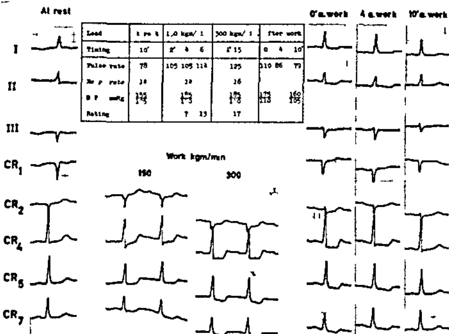


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The final criteria for selection were a normal or only slight increase in heart volume absence of symptoms of decompensation arterial hypertension of a degree demanding treatment signs of recent infarction or of other complicating disease In all cases the clinical course of the disease had been

TABLE I Intravenous injection trial Results obtained during exercise test and their statistical treatment

Comparison is made between the different Ugontin preparations (A = oxyethyltheophylline D = Segontin E = placebo) and between the injections in their chronological order Mean values of the 10 patients are given for W_{max} time lag of onset of precordial pain and the heart rate during the second work load The variance of the differences between the 3 preparations and the 3 injections in their chronological order is compared with the error (residual variance)

	Control test	Ugontin			Variance compared with error	Injection no			Variance compared with error
		A	D	E		1	2	3	
W_{max} (kpm/min)	494	518	517	521	v<e P<0 05	520	520	516	v<e P<0 05
Time lag of onset of precordial pain (min)	12 5	12 4	12 6	12 9	v<e P>0 05	12 6	12 5	12 8	v<e P>0 05
Heart rate	114	112	110	114	v>e P>0 05	112	111	113	v e P 0 05

TABLE II Oral administation trial Results obtained during exercise test and their statistical treatment

Comparison is made between the different Ugontin preparations (B = oxyethyltheophylline V = Segontin S = placebo) and between the different periods in chronological order For further explanation see table I

	Control test	Ugontin			Variance compared with error	Period no			Variance compared with error
		B	S	V		1	2	3	
W_{max} (kpm min)	494	556	536	516	v<e P>0 05	537	538	533	v e P 0 05
Time lag of onset of precordial pain (min)	*12 5*	*14 1*	*13 5*	*14 0*	v<e P>0 05	*13 4*	*14 0*	*14 2*	v e P 0 05
Heart rate	114	116	116	113	v<e P>0 05	118	112	114	v e P 0 05

ference was not significant (P > 0 7) Thus even a training effect of some significance induced by the repeated exercise tests can be excluded

Oral administration trial

Considering both Ugontin B S and V and the different chronological periods 1 2 and 3 the variance of the differences

TABLE III Oral administration trial Comparison between the entire periods with regard to symptoms and nitroglycerine consumption and statistical treatment by χ^2 analysis

Comparison is made with regard to the different Ugontin preparations and the different periods in their chronological order Mean values are given for the number of anginal attacks the number of nitroglycerine tablets consumed and the patients evaluation of the preparation s effect on their condition (much improved = +2 slightly improved = +1 unchanged = 0 slightly worse = −1 much worse = −2)

	Ugontin				Period no			
	B	S	V	χ^2	1	2	3	χ^2
Anginal attacks no	232	215	234	P > 0 5	264	232	191	P < 0 05
Nitroglycerine tabl no	129	80	102	P > 0 05	155	69	87	P < 0 001
Effect	+0 5	+0 9	+1 0	P > 0 5	+0 7	+0 9	+0 8	P > 0 05

of the results obtained during the exercise test was not significant Values for W_{max} the time lag of precordial pain and the heart rate are given in table II The variance of the differences between the periods 1, 2 and 3 for W_{max} is significantly less than the error

Neither was there a significant difference between Ugontin B, S and V concerning pain attacks, consumption of nitroglycerine tablets and the patients judgement of the preparations effect (table III) Concerning the different periods in their chronological order however, a significantly decreasing frequency of pain attacks was observed This holds for the complete periods as well as for the last week of each period ($P < 0\ 05$) Concordantly, consumption of nitroglycerine tablets was significantly higher during the first than during the two last periods No difference was noticed, however, as to the patients judgement of the preparations effect

The only side effect noticed was a slight drowsiness described as not unagreeable" in one patient

ECG analysis

As shown in table IV no significant difference seems to exist between the different preparations regarding their effect on the ECG at rest and during exercise This holds for both the intravenous and the oral trial

Discussion

This double blind trial revealed no statistically significant differences between the effect of oxyethyltheophylline Segontin or placebo, regarding neither the patients' subjective symptoms nor the ECG or other functional parameters measured during the exercise tolerance test This result disagrees with earlier reports of the beneficial effect of Segontin in angina pectoris, and also with our own experience (KAJ, MLL) in clinical trials without a blind test One possible explanation may be the small number and the method of selection of our case material, taking only male patients without hypertension or heart decompensation

TABLE IV ECG at rest (r) and during work (w) at different trial periods compared with the ECG of the initial control test

	Intravenous administration						Oral administration					
	A		D		E		B		S		V	
Patient	r	w	r	w	r	w	r	w	r	w	r	w
KB	0	0	0	0	0	0	0	0 VPB	+1	0	+1	0
GS	0	0	0	0	0	0	0	−1 VPB	0	−1 VPB	0	−1 VPB
ID	0	+1	0	+1	0	0	0	−1	0	0	0	0
EH	0	0	0	0	0 VPB	0	+1	0	+1	0	0	0
KO	0	0	+1	0	0	0	−1	0	0	0	0	0
AE	0	0	0	+1	0	0	0	0	0	0	0	0
BK	0 VPB	0	0	+1 VPB	0	0	+1	0	0	0	0	0
OB	0 AVI	0	0	0	0	0	+1	+1	0	0	0	0
BJ	0	0	0	0	0	0	0	0	+1	0	0	0
TF	+1	0	0	0	0	0	0	0	+1	0	0	0
Total	+1 VPB AVI	+1	+1	+3 VPB	0 VPB	0	+2	−1 VPB	+4	−1 VPB	+1	−2 VPB

+1 = ECG improved
0 = ECG unchanged
−1 = ECG more pathological
VPB = Occurrence of ventricular premature beats
AVI = Occurrence of first degree atrioventricular block

With regard to evaluation of treatment of angina pectoris it is noteworthy that the anginal attacks decreased during consecutive periods independent of the drug given and that nitroglycerine consumption was lower during the last two periods In earlier reports this phenomenon may have contributed to the favourable clinical results obtained with Segontin It is remarkable that this improvement in subjective angina pectoris was not paralleled by any objectively measurable improvement during the exercise test, which showed W_{max}, the time lag of onset of precordial pain and the heart rate to be mainly unchanged The subjective improvement may be explained either by a *placebo effect*, which of course does not influence behaviour during the work test, or by the patients gradual adaptation to the disease learning to avoid situations during every day life which may evoke angina This would be supported by the

changed perception of precordial pain and its time lag during the work test. This experience stresses the importance that drugs, whose effects are to be compared, should be given in varying sequence during a comparative study.

Conclusion and summary

A double blind trial using prenylamine (Segontin), oxyethyltheophylline and placebo was made in 10 specially selected male patients with ischaemic heart disease and typical angina pectoris. The drugs were administered intravenously in one single test dose and orally in periods of 4 weeks. Evaluation of their effect was based on 1) results obtained during exercise tolerance tests including ECG recording, 2) the incidence of anginal attacks and the consumption of nitroglycerine. The results were analysed with regard to both the different preparations and the different periods of administration. No statistically significant differences were observed between the different preparations. The frequency of angina pectoris however, decreased significantly during consecutive periods of observation. It is concluded that the gradual adaptation to the disease and/or a pure placebo effect had more influence on perception of angina pectoris than any of the preparations tested.

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From the Medical Department VII (Head E M Blegen M D) Ullevål Hospital Oslo Norway

Serum α-hydroxybutyric Dehydrogenase in Myocardial Infarction

By

KNUT JOACHIM BERG, ERIK ENGER and HANS BENESTAD

The major disadvantage using enzyme values in the diagnosis of myocardial infarction is the lack of specific heart enzymes An increase in the serum activities of glutamic oxalacetic trans aminase (GOT) and lactic dehydrogenase (LDH) can only be taken as evidence of myocardial damage when other causes are excluded (23) GOT is reported to be elevated in 97 per cent and LDH in 86 per cent of patients with myocardial infarction (1) Such figures are probably too high It is difficult to detect small infarctions by clinical methods and uncertain cases are frequently omitted from published re ports The relatively short duration of the GOT elevation restricts the practical application of this enzyme in the diagnosis of myocardial infarction The eleva tion of LDH persists longer than GOT and is therefore preferable when the determination has to be made several days after the acute attack

Studies on LDH have shown at least 5 isoenzymes with different chemical physical antigenic and enzymatic properties On this basis electrophoretic methods (15 25), chromatographic meth ods (8, 10) and separation procedures depending on different degrees of heat stability (2) have been devised for identification Different organs seem to have more or less typical patterns of LDH isoenzymes Elevation of the electrophoretically fast moving LDH isoenzyme in serum ("the heart frac tion) may reflect minor degrees of myocardial necrosis despite normal LDH values (6, 12) In myocardial infarction the heart fraction is often elevated several weeks after total LDH is normal ized (6 25) LDH isoenzyme determina tions are therefore of considerable value in the diagnosis of myocardial infarction So far however the separa tion procedures are too laborious for routine clinical use

In 1950 Meister (11) demonstrated that LDH not only reduces pyruvate to lactate but also reduces several diketo-acids the effect on α ketobutyric acid

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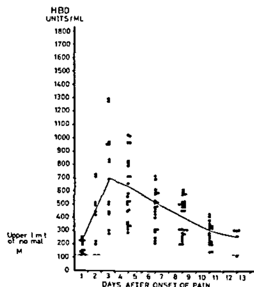


Fig 1 Serial HBD determinations in 28 patients with myocardial infarction

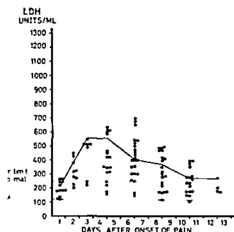


Fig 2 Serial LDH determinations in 28 patients with myocardial infarction

being particularly marked Rosalki and Wilkinson (18) and Elliot and Wilkinson (4) showed that the heart fraction of LDH had a greater enzymatic effect on the *a* ketobutyric acid than on pyruvic acid They called the enzyme when the former substrate was used *a hydroxybutyric acid dehydrogenase* (HBD) The difference between HBD and LDH may be related to a shift in the LDH isoenzyme pattern (22) It has been maintained by several authors that HBD is a more specific heart enzyme than LDH and that it remains elevated for a longer time than LDH after an acute myocardial necrosis (5, 9, 16, 19, 20, 21)

The HBD/LDH ratio is reported to be high in myocardial disease and low in liver disease (3, 9, 14, 16, 19), although there is no general agreement on this point (7, 13)

This paper is concerned with the relative diagnostic value of LDH and HBD by serial studies of 28 patients with myocardial infarction

Material and methods

The material consists of 28 patients (23 men and 5 women) with myocardial infarction The mean age was 59 years (range 39 to 72 years) Twenty five patients experienced their first and 3 patients their second infarction

The diagnosis was based on the clinical picture on typical electrocardiographic changes in the 12 conventional leads on leucocytosis increased ESR and fever The diagnosis was made without considering the enzyme analyses In 27 patients the acute infarction occurred within 48 hours before the first blood sample was drawn while 3 or 4 days elapsed in the last patient The first blood sample was taken immediately after admission Subsequently determinations were made daily for 3 days on fasting morning samples During the next 10 days this was done every second day Serum was separated from cells without delay and stored at — 18 C The enzyme activity was determined within 1 week usually on the same day Care was taken to ensure that no change in enzymatic activity occurred during storage

TABLE I Percentage of pathological enzyme values

	Days after infarction								
Enzyme	1	2	3	4	5	6–7	8–9	10–11	12–13
LDH	40	74	89	93	93	89	67	58	28
HBD	47	95	96	96	96	89	85	78	56
GOT	47	92	96	95	68	37	26	16	4

for one week All three enzymes were analyzed in the same sample

Enzymatic activity of LDH and HBD was determined using reagents from Sigma LDH was measured as described by Wroblewsky and La Due (24) and HBD by the spectrophotometric method of Elliot and Wilkinson (4) Parallel measurements of LDH and HBD were made by the same person always using the same DPNH solution at the same temperature As far as possible the use of temperature corrections was avoided GOT was determined by an auto analyzer at the Central Laboratory Ullevål Hospital by Reitman and Frankel s colorimetric method (17) The results are expressed in conventional spectrophotometric units 1 spectrophotometric unit being equal to 0 48 international units

Normal levels of HBD and LDH were obtained by analyzing sera from 32 blood donors The upper normal limit of HBD was 225 units/ml (mean value 126 units/ml range 48 to 212 standard deviation 33) The upper limit of LDH was 250 units/ml (mean value 130 units/ml range 54 to 229 standard deviation 41) The upper normal limit of GOT was 36 units/ml (mean value 18 units ml standard deviation 6) based on blood donors investigated at the Central Laboratory

Results

Figs 1 and 2 give the post infarction values of HBD and LDH HBD was elevated more often showed a higher mean value than LDH and the elevation lasted longer

Table I presents the percentages of pathological LDH, HBD and GOT values on each day after the infarction During the first 3 post infarction days these values were approximately the same for HBD and GOT, although the relative elevation of the mean GOT values was most prominent However, the mean HBD value remained elevated for more than a week after GOT had returned to normal HBD was pathological at least in one determination in each patient LDH failed to exceed the normal range in 2 patients and GOT in 1 patient The latter was admitted to

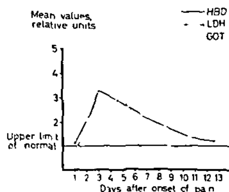


Fig 3 Comparison of mean values (relative units of HBD LDH and GOT in 28 patients with myocardial infarction

hospital 3 or 4 days after the acute attack.

Fig. 3 compares the mean values of LDH, HBD and GOT expressed in relative units (mean value/upper normal limit). The slopes of HBD and LDH were practically the same with slightly but consistently higher mean HBD values. The mean HBD/LDH ratio was over 1 on each day (grand mean value 1.17 against 0.97 in the normal material). In a preliminary study 10 patients with hepatitis and 10 patients with occlusive jaundice were studied. The mean HBD/LDH ratio in the former was 0.71 and in the latter 0.97.

Comments

The study clearly demonstrates the diagnostic value of enzyme determinations in myocardial infarction. HBD showed an earlier and more pronounced elevation than LDH but this difference is probably of minor practical importance. During the subsequent course the curves of their mean values showed a parallel decline. At the end of the observation period (12 to 13 days) the mean value of both enzymes was still outside the normal range, HBD exhibiting a greater number of pathological values than LDH. At this stage however only a limited number of observations was made. Serial LDH determinations failed to exceed the upper normal limit in 2 patients whereas at least one pathological HBD value was obtained in each individual. The explanation for these small, but easily demonstrable, differences between LDH and HBD is probably that the latter to a greater extent reflects the fast moving heart fraction of the LDH isoenzymes which is reacting more specifically with butyrate than pyruvate. Thus the two enzymes probably represent the same activity, HBD being slightly more sensitive than LDH. The determination of HBD should therefore replace total LDH in the diagnosis of myocardial infarction. This study was not primarily concerned with the organ specificity of the two enzymes. However, another argument for the use of HBD was the finding of a greater elevation of LDH than HBD in patients with hepatitis and occlusive jaundice.

Rosalki and Wilkinson (19) have maintained that HBD might even replace the laborious separation of the organ specific LDH isoenzymes. This is a dubious statement. In the case of a particularly small myocardial necrosis and if uncertainty exists whether an enzyme elevation is related to heart or liver disease, we think that the separation of LDH isoenzymes is still necessary.

The study demonstrates the value but also the limitations of serial GOT determinations in myocardial infarction. This enzyme showed an early and pronounced elevation which clearly exceeded that of HBD. Although the lack of specificity and the transient character of the GOT rise are limiting factors, this enzyme is still very useful during the acute stage of the illness. If the patient is studied several days after the onset of chest pain HBD determinations have their great advantage. Thus determinations of GOT and HBD are reliable and supplementary methods in the diagnosis of acute

myocardial infarction If only one enzymatic method has to be chosen HBD is preferable until simple methods for the determination of LDH isoenzymes are developed

Summary and conclusions

In 28 patients with clinical and electro cardiographic evidence of acute myo cardial infarction serial determinations were made of GOT HBD and LDH during the first 13 days HBD showed at least one pathological value in each patient whereas LDH failed to exceed the normal range in 2 patients and GOT in one During the initial phase GOT and HBD exhibited pronounced rise, that of GOT being most prominent In contrast to GOT HBD and LDH showed pathological values for a prolonged period At the end of the observation their mean values were still above the upper normal limit HBD should replace total LDH in the diagnosis of myocardial infarction until methods for separation of the LDH isoenzymes suitable for routine clinical use are developed

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From the Department of Internal Medicine (Head Professor E Ask Upmark M D)
University Hospital Uppsala, Sweden

Milk-induced Colitis

By

Sven Mårten Samuelsson and Ivar Werner

Milk allergy has been connected with many symptoms in infancy, for instance vomiting, colic, diarrhoea, eczema, thrombocytopenia, respiratory and cerebral syndromes (2, 7 10 15) It is commonly stated in paediatric literature that the frequency is highest during the first two to three years of life and then gradually decreases (7, 10) The actual incidence of milk allergy is not known however The figures found in the literature vary greatly depending e g on the various diagnostic criteria used and the frequency of allergy in the population examined (10) In large series of unselected healthy children the incidence of cow s milk allergy has been estimated as 0 3—1 3 % (10) In a series of allergic children an incidence of up to 30 % has been reported (2) Two and a half per cent of patients with known cow s milk allergy are still sensitive to milk after the age of six years (7) Cases of milk allergy in adults are seldom reported

A case of milk induced colitis in a sixteen year-old boy has recently been studied in the Medical Department and as it showed certain points of interest it is reported here

Case report

G E 2266/62 No allergy was known in the family The patient had two healthy siblings Delivery was normal. The birth weight was 4 300 g During the first 2 1/2 months of life he was fed with his mother s milk Then a small amount of cow s milk and wheat flour was added to the diet At the age of 3 months he had frequent watery and often blood stained stools — 4—5 each day and 2—3 each night — which continued with great intensity during the first two years of life He also had recurrent periods of fever One year old he had frequent upper respiratory infections At the age of two years an anaemia with haemoglobin 7 0 g % was found The stools showed a positive benzidine reaction He was treated with two blood transfusions During the following years he had 3 to 4 watery stools every day He had difficulty in gaining weight At the age of four sigmoidoscopy was normal At eleven his stools still contained traces of blood sigmoidoscopy showed a somewhat granular mucosa X ray examination showed a somewhat deficient haustration of the descendent colon and sigmoid

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At 15 he developed an upper respiratory tract infection after which his stools became more frequent and associated with colic His appetite was poor Treatment with Salazosulphapyridine, chlorchinaldolum and a diet without vegetables had no effect

In 1962, at the age of 16 he was admitted to the Medical Department of the University Hospital On examination he was tall (176 cm) and very thin (44 kg) The physical examination was in other respects normal Laboratory examination showed an iron deficiency haematocrit 38—40 %, serum iron about 38 and total iron binding capacity about 380 μg/100 ml There was no eosinophilia of the blood The stools contained no blood There was no steatorrhoea The gastric juice contained free hydrochloric acid Oral glucose and xylose tolerance tests were normal There was a normal concentration of sodium chloride in the sweat Serum alkaline phosphatase was 4 2—7 6 B L units (upper normal limit 2 5 B L units in adults) Serum cholesterol was about 107 and total lipids about 450 mg/100 ml X ray examination of the stomach and small intestine was normal Barium enema showed a somewhat scanty and irregular haustration of the whole colon No ulceration was visible In the lower descending colon the mucosal pattern was changed showing mucosal wrinkles lengthways Sigmoidoscopy was almost normal with a very slight granulation No ulceration was seen Rectal biopsy showed slight oedema and congestion in the superficial parts of the mucosa Lymphocytes and plasma cells were moderately increased in number outside the lymph follicles of the mucosa Biopsy from the jejunum with a Crosby capsule showed a normal histological picture

Undiluted serum contained precipitins to whole cow s milk The precipitins were not found in diluted serum Intradermal test with cow s milk was negative

On the probable diagnosis of milk induced colitis the patient was treated with a milk free diet There was a prompt improvement On admission he had 3—4 watery mucous stools each day On the milk free diet the stools were quite normal within three weeks He gained 6 kg in six weeks

In order to test the possibility of intestinal lactase deficiency he was given a lactose load on two different occasions No apparent disturbance of the intestinal functions was observed

At home he has continued with the same diet He has grown 4 cm His weight has increased to 56 kg His stools have remained normal On occasional digressions from the diet he has had 1—2 loose stools per day

Case summary A 16 year old boy with a history of watery and sometimes bloody stools since the age of 3 months The examination of the colon showed a slight abnormality but not the picture characteristic of ulcerative colitis Precipitins to cow's milk were found in the blood There was no lactose intolerance On a milk free diet the stools became normal and have remained normal There was a rapid gain of weight

Discussion

It is often difficult to prove the existence of cow s milk allergy in a given case Different approaches to the problem have been suggested According to most authors skin tests with milk proteins are without diagnostic value Yet, strongly positive skin reactions may occur only in milk allergic patients Other procedures such as demonstration of eosinophils in peripheral blood, nasal, bronchial or intestinal mucus are also of doubtful value The basophil reduction test however, seems to be of significance (16) In a case of milk allergy there was a

marked decrease in circulating basophils and their granulation after provocation with milk (16)

Clinically many symptoms have been associated with milk allergy mostly in children One group consists of usual allergic symptoms for instance eczema, urticaria rhinitis and asthma These symptoms often disappear when milk is withdrawn from the food and reappear on reintroduction of the milk (2, 10) Thus Colldahl recently demonstrated that whole milk and certain milk products may cause asthmatic attacks in adult patients (8) The patients improved on an elimination diet

A second group of symptoms consists of acute anaphylactic reactions occurring in infants under the age of one year often with a fatal termination called cot death Collins Williams has collected 29 such cases from the English literature (9)

A third group consists of symptoms from the gastro intestinal tract, mostly diarrhoea (2, 14) but there may be a variety of other symptoms for instance refusal of milk vomiting colic pains (20) pylorospasm and signs of coeliac disease (23)

A fourth group of symptoms has recently been associated with milk allergy It constitutes a syndrome of poor weight gain gastrointestinal and upper respiratory tract symptoms — such as vomiting diarrhoea protein losing enteropathy chronic rhinitis recurrent upper respiratory tract infections — pulmonary haemosiderosis and iron deficiency anaemia (15) It occurs essentially in infants By means of a Cr 51-labelled erythrocyte technique some of these patients with hypochromic microcytic anaemia have been shown to lose significant amounts of faecal blood when fed with homogenized milk (26) The faecal blood loss was decreased during periods of ingestion of a soybean or heat processed cow s milk formula Several cases have been found to have circulatory precipitating antibodies to milk proteins (15, 26)

Rowe et al reported 26 cases of diarrhoea caused by food allergy of which milk, fruits and spices were the most frequent sources (21) The authors stressed that the patients were adults even more than 50 years old Similar findings in adults have been reported by Friedenwald and Morrison (13) and Cardon (5) but the reports are few In the majority of cases belonging to this third group direct evidence of allergy is often lacking The patients have more or less completely recovered on the withdrawal of milk from the food The term "milk allergy should in these cases be changed to "milk intolerance

Milk has sporadically been considered as a causative factor of ulcerative colitis first suggested by Andresen in 1925 (1) In 1942 Andresen could show that milk was an important allergic factor in 13 out of 50 cases of ulcerative colitis (1) Mackie found clinical evidence of food allergy in 44 and possible evidence in seven out of 67 cases of ulcerative colitis (17) Milk eggs oranges wheat, spinach and tomatoes named in order of frequency headed the list of food allergens

Using elimination diets complete relief of symptoms was obtained in seven of 14 cases of ulcerative colitis by Rowe (20) In six of the cases specific foods re

produced diarrhoea Milk headed the list of allergenic foods In 1961 Truelove documented a clinical relationship between cow s milk and ulcerative colitis in 13 out of a series of more than 200 patients (25) Removal of milk and proteincontaining milk products from the diet was followed by a marked improvement In five of the patients, milk was reintroduced into the diet resulting in a relapse The interval before the return of the colitis was proportional to the length of the remission In a recent report by Wright and Truelove a series of patients with ulcerative colitis was given a milk free diet over a trial period of one year (28) Another series was given an ordinary diet Ten patients on the milk free diet were symptom free throughout the trial period against five patients on the ordinary diet The authors estimated that a milk free diet would be beneficial to about one in *five patients with ulcerative colitis* They suggested that the proportion might be higher in patients in their first attack of the disease Sewell et al found milk intolerance in 26 cases of different gastrointestinal disorders, among them in 3 cases of ulcerative colitis (23)

A severe case of ulcerative colitis of 11 years duration was recently reported by Citrin (6) The proctoscopic and colonic X ray pictures were typical of the advanced disease By omitting milk from the diet the patient recovered completely The proctoscopic examination showed no evidence of colitis and the colonic X ray pictures were quite normal 15 months after starting the diet

Edlen and Heimdal treated 23 cases of ulcerative colitis with a basic diet 'free from antigenic properties" (12) In all the cases there was an intolerance against milk and milk products 13 of the cases had no relapse Provocation with milk resulted in an immediate relapse in seven cases There was, however, no report on the extent and severity of the disease in the different cases and the results of the follow up investigations were not reported The opinion of these authors that nearly every case of ulcerative colitis might be cured by diets free from milk or some other food stuffs is contrary to the findings of others Truelove is of the opinion that milk allergy plays a significant role in only a small proportion of the patients with ulcerative colitis (25, 28) This opinion is in agreement with the results from the Medical Department of Uppsala We have been treating about 150 patients with ulcerative colitis during the last ten years Many of these patients have *improved but none has been completely* cured by omitting milk from the diet

It must be stressed that disappearance of symptoms when milk is withdrawn from the diet does not necessarily mean milk allergy Other disorders must be excluded, for instance galactosaemia and intestinal lactase deficiency Milk may contain penicillin or other contaminants (10), which may produce allergic manifestations not caused by the milk itself In a series of 382 cases of milk allergy" only 1 % was hypersensitive to the milk proteins, the other 99 % were allergic to allergens of bacteria or other contaminants in the milk (27)

The problem of milk allergy may also be approached from immunological aspects The results are, however, dif

ficult to evaluate It is known since the beginning of this century that cow s milk proteins are highly antigenic In 1906 Moro demonstrated cow s milk protein and milk precipitins in the blood of an atrophic infant (18) In 1916 Schloss and Worthen showed that un digested or partly digested proteins for instance from cow s milk could be ab sorbed in patients with nutritional or gastro enteric disorders and also in very young infants (22) In later reports they showed that the blood of many marasmic infants contained precipitins to cow's milk during some period of the disease During the course of diarrhoea it was possible to demonstrate the absorption of antigenic cow s milk protein and later to observe the appearance of precipitins to cow's milk in the blood Precipitating antibodies persisted for months in the marasmic infants whereas in normal infants the degree of precipitin forma tion was relatively slight and the precipi tin could be demonstrated in the blood only for short periods

By different methods, for instance complement fixation test, tanned red cell haemagglutination test of Boyden (19) diffusion in gel and double dif fusion in gel test (15) immunoelectro phoresis (15) cutaneous anaphylaxis reaction in guineapigs and examination of specifically bound radio iodinated serum proteins many healthy subjects (newborn infants children and adults) have been found to have serum anti bodies to cow s milk (4, 19)

Milk antibodies have been found in a large proportion of eczematous and dyspeptic infants Complement fixing serum antibodies have often been meas urable in higher dilutions in these children than in normal infants

Gunther et al found circulating milk antibodies in 98 % of a group of normal infants by the coated tanned red cell technique The antibodies were specific for casein, alpha lactalbumin and bovine serum albumin From such findings they assumed that 'cot death might result from anaphylaxis following inhalation of cow s milk by a sensitized sleeping baby They obtained experimental support for their hypothesis in guinea pigs sensitized to cow s milk The animals died when a drop of milk was placed in the larynx Autopsy revealed pulmonary changes seen in human cot death Some other authors have however failed to find milk antibodies in children who have died cot deaths (19)

Milk antibodies have been found in some cases of assumed intestinal milk allergy or gluten induced coeliac disease (23) One case was tested repeatedly and when the complement fixing anti bodies disappeared tolerance of cow s milk was achieved

A few reports on the occurrence of milk antibodies in ulcerative colitis have appeared Taylor and Truelove studied the occurrence of antibodies to purified cow s milk proteins in 75 cases of ulcerative colitis and 50 healthy subjects by the coated tanned red cell technique (29) More than five times as many patients with ulcerative colitis (32 patients) showed a reaction at a titre of 1/20,000 for any of the proteins tested as did normal subjects (4 subjects) No correlations were found between various clinical features of the patients with ulcerative colitis — for instance with

produced diarrhoea Milk headed the list of allergenic foods In 1961 Truelove documented a clinical relationship between cow's milk and ulcerative colitis in 13 out of a series of more than 200 patients (25) Removal of milk and proteincontaining milk products from the diet was followed by a marked improvement In five of the patients, milk was reintroduced into the diet resulting in a relapse The interval before the return of the colitis was proportional to the length of the remission In a recent report by Wright and Truelove a series of patients with ulcerative colitis was given a milk free diet over a trial period of one year (28) Another series was given an ordinary diet Ten patients on the milk free diet were symptom free throughout the trial period against five patients on the ordinary diet The authors estimated that a milk free diet would be beneficial to about one in five patients with ulcerative colitis They suggested that the proportion might be higher in patients in their first attack of the disease Sewell et al found milk intolerance in 26 cases of different gastrointestinal disorders among them in 3 cases of ulcerative colitis (23)

A severe case of ulcerative colitis of 11 years duration was recently reported by Citrin (6) The proctoscopic and colonic X ray pictures were typical of the advanced disease By omitting milk from the diet the patient recovered completely The proctoscopic examination showed no evidence of colitis and the colonic X ray pictures were quite normal 15 months after starting the diet

Edlen and Heimdal treated 23 cases of ulcerative colitis with a basic diet free from antigenic properties (12) In all the cases there was an intolerance against milk and milk products 13 of the cases had no relapse Provocation with milk resulted in an immediate relapse in seven cases There was, however, no report on the extent and severity of the disease in the different cases and the results of the follow up investigations were not reported The opinion of these authors that nearly every case of ulcerative colitis might be cured by diets free from milk or some other food stuffs is contrary to the findings of others Truelove is of the opinion that milk allergy plays a significant role in only a small proportion of the patients with ulcerative colitis (25, 28) This opinion is in agreement with the results from the Medical Department of Uppsala We have been treating about 150 patients with ulcerative colitis during the last ten years Many of these patients have improved but none has been completely cured by omitting milk from the diet

It must be stressed that disappearance of symptoms when milk is withdrawn from the diet does not necessarily mean milk allergy Other disorders must be excluded, for instance galactosaemia and intestinal lactase deficiency Milk may contain penicillin or other contaminants (10), which may produce allergic manifestations not caused by the milk itself In a series of 382 cases of ' milk allergy only 1 % was hypersensitive to the milk proteins, the other 99 % were allergic to allergens of bacteria or other contaminants in the milk (27)

The problem of milk allergy may also be approached from immunological aspects The results are, however dif

cow s milk was added to the diet in early childhood and disappeared when milk was omitted 16 years later There were no signs of lactase deficiency Milk antibodies in a low titre were found in the blood of the patient The literature on milk allergy is discussed from clinical and immunological aspects The true incidence of milk allergy is not known The relationship between milk and ulcerative colitis is discussed It is stressed that milk allergy is probably not of primary aetiological importance in the development of this disease Secondarily, however, milk allergy may develop Many patients improve on a milk free diet but they are seldom or never completely cured

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From the University Clinic of Internal Diseases Amsterdam (Head J G G Borst M D) and from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Head J J van Loghem M D) Amsterdam, the Netherlands

Urokinase Excretion in Patients with Renal Diseases

By

J VREEKEN, J BOOMGAARD and K DEGGELLER

Opinions differ about the origin of urokinase, a plasminogen activator found in the urine It was thought (11, 20) that urokinase is the plasma plasminogen activator which is excreted in urine As previously described (5) we could not find a relationship between urokinase excretion and fibrinolytic activity of blood Urokinase may also be a product of the uropoietic system itself A plasminogen activator was found by Barnett and Baron (1) in monkey renal tissue cultures, by Prokopowicz et al (19) in dog kidney tissue by Kwaan and Fischer (14) in the kidney tissue of man and different animals while Brakman and Astrup (6) showed that the fibrinolytic activity of the euglobulin fraction of venous blood of the kidney was higher than that from the artery Painter (17) demonstrated an accumulation of a soluble plasminogen activator during the growth of tissue cultures of monkey and dog kidney cells in serum free media and not with cultures of cells of other organs This activator resembles urokinase in its electrophoretic properties on starch urea gels Bjerrehuus (2) observed that fibrinolytic activities in the urine of the bladder were the same as those obtained from the renal pelvis Holemans and Johnston (10) studying the isolated dog kidney suggested that the amount of activator released was a function of the endothelial surface of the vessel walls

From these observations it seems very likely that, if urokinase is produced in the urinary system the kidney itself will be the site of origin To study this possibility urokinase excretion was determined in patients with renal diseases and correlated with renal function

As described previously renal tubules in all probability have no influence on the urokinase excretion (5) We therefore were especially interested in the glomerular function of the patients This was measured by means of the endogenous creatinine clearance (15)

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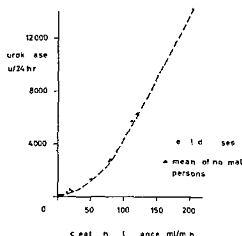


Fig 1 The correlation between creatinine clearance and urokinase excretion in all kind of renal diseases

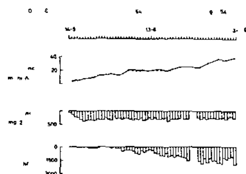


Fig 2 Simultaneous rise of creatinine clearance and urokinase excretion after acute glomerulonephritis

Materials and methods

Urokinase assay Urokinase activity was determined by measuring the lysis times of standard fibrin clots containing dilutions of a urine or urokinase standard preparation as previously described (4) The 24 hours urokinase excretion (with the standard deviation) in normal women amounts to 5 770 ± 1,420 units in normal men 6 800 ± 1 520 units The units are according to Ploug and Kjelgaard (18)

Creatinine This was measured according to De Vries and Van Daatselaar (21)

Urokinase determinations were performed in patients with all kinds of kidney diseases No patients with essential hypertension were included in the present study From patients in a steady state of their illness the 24 hours urokinase excretion was calculated as the average of the determinations on at least three days The creatinine excretion was always taken as a measure for an adequate collection of the 24 hours urine

Results

In general a correlation was found between glomerular function, as measured by creatinine clearance, and urokinase excretion regardless of the kind of kidney disease (fig 1) When the creatinine clearance was decreased the urokinase excretion was lowered as well In 4 patients with nephrotic syndrome and a supranormal creatinine clearance the urokinase excretion was also above the normal limits Although the fluctuations in the 24 hours urokinase excretion of patients in a steady state of renal disease were greater than in normal persons, the excretion tended to be constant just as they were in normal persons (3) In nearly all patients with a changing creatinine clearance during the observation period the correlation between creatinine clearance and urokinase excretion was also demonstrable

Of a patient (state No 64086) with acute glomerulonephritis, the results of the observations are recorded in fig 2 In this patient after a period of near anuria creatinine clearance and urokinase excretion rose simultaneously This patient did not completely recover and urokinase excretion like creatinine

clearance remained below normal In a patient with periureteritis fibrosa creatinine clearance increased from 4 ml before to 50 ml per minute after the ureters were surgically freed from the fibrotic process In accordance with this, the urokinase excretion increased *from undetectable before, to 2,250 units/* 24 hours after the operation

Discussion

In patients suffering from renal diseases in general a correlation could be established between renal function as measured by endogenous creatinine clearance and urokinase excretion whether these patients had a constant or a changing creatinine clearance during the observation Smyrniotis et al (20) Den Ottolander and Bleyenberg (16) and Edward et al (8) could not detect urokinase in the urine of uraemic patients A correlation, however be tween urokinase excretion and creatinine clearance was as yet not mentioned in the literature When considering this diminished urokinase excretion in the presence of decreased glomerular filtra tion the following explanations can be put forward

1 Urokinase is produced in the kidney and the same pathology which is re sponsible for the diminished kidney function is also the cause of the diminish ed production and excretion of uro kinase

2 Urokinase is the plasma plasmino gen activator which is poorly excreted because of diminished filtration by the glomeruli which are diseased or diminish ed in quantity

In considering the last possibility it has to be pointed out that, with some other substances which are retained because of pathological glomerular func tion, excretion finally comes into balance with production in the presence of higher circulating levels of these sub stances *Failure to reach eventual equilib* rium is incompatible with life When urokinase is the excreted form of plasma plasminogen activator, some other ex planation therefore must exist for the constantly decreased urokinase excretion in the steady state of renal diseases It can be supposed that there are other mechanisms of urokinase elimination in cases of diminished glomerular filtration or one can suggest a compensatory fall in production in those situations No indications are present for a high cir culating level of plasma plasminogen activator in subjects with depressed glomerular filtration In uraemic pa tients with haemorrhagic diathesis, no altered fibrinolytic activity of the blood was established (7) The suggested other than renal mechanisms for the elimination of urokinase must be so effective and in such close correlation with the renal function that detectable blood levels never exist A diminished urokinase excretion in patients with a diminished glomerular filtration seems for all those reasons more likely to be caused by the diminished kidney func tion than by a diminished filtration of plasma plasminogen activator

Our observations in normal persons that urokinase excretion and fibrinolytic activity in the blood were unrelated also suggested that urokinase is not the excreted plasminogen activator from

the blood (3) The correlation between urokinase excretion and endogenous creatinine clearance in renal diseases and the constantly diminished urokinase excretion in patients with diminished kidney function are best explained by accepting the glomeruli as production place of urokinase

Since plasma plasminogen activator in all probability is produced in the vascular wall (12, 13, 10) it seems likely that this is also the glomerular source of urokinase In normal circumstances this activator will then be transported with the glomerular filtrate to the urinary tract

Although fig 1 shows a clear correlation between urokinase excretion and creatinine clearance this correlation does not appear to be linear all over the curve has a "bend" in the low ranges Besides this it does not go straight through the origin The bend means that the urokinase excretion is already unmeasurable when the creatinine clearance is below $\pm$ 10 ml/minute Though the creatinine clearance — especially in the low range — does not exactly reflect the glomerular filtration rate, the discrepancy between urokinase excretion and creatinine clearance in this area is greater than can be explained from inexactitude of the creatinine clearance alone (e g the real glomerular filtration rate at a level of 10 ml/min creatinine clearance may be lower but not zero as the urokinase excretion is)

The lack of urokinase in urines from patients with a creatinine clearance lower than 10 ml/min may be caused by insensitiveness of the urokinase determination in this area, but this cannot account for the shape of the curve in the higher regions We never found evidence of urokinase inhibiting substances in uraemic urines (4)

The hypothesis that urokinase production in renal diseases is more rapidly diminished than the filtration rate is less attractive as we find this phenomenon regardless of the kind of kidney disease Another way of renal handling of urokinase when the kidney function is diminished is a possibility, but for us perhaps the best explanation is found in the idea that urokinase is produced in the glomerular walls and that it correlates with filtration surfaces, but that even with clearly diminished filtration surface the filtration rate itself can be kept up to the mark for a long time by the influence of filtration pressure

Summary

1 In patients suffering from all kinds of renal diseases there is in general a correlation between renal function as measured by creatinine clearance and urokinase excretion

2 In all probability urokinase is not the plasminogen activator excreted from the blood The given facts are best explained by the hypothesis that the glomerulus is the place of production

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From the Division of Nephrology, Medical Department P (Head J Hess Thaysen) Rigshospitalet (University Hospital) Copenhagen Denmark

Pronounced Polyuria and Natriuresis Following Surgical Relief of Urinary Tract Obstruction

By

I STEINESS and M LUNDING

In 1951 Wilson et al (8) described three patients in whom relief of a urinary tract obstruction was followed by so copious an excretion of sodium and water as to endanger life because of extracellular dehydration The state was reversible

In a clearance study of four patients with a similar disturbance in renal function following the institution of drainage, Bricker et al (1) found that the abnormality was due to a suppression of the reabsorption of sodium in the proximal tubules In contrast, Maher et al (3) encountered a case of massive post obstructive diuresis where the polyuria was attributed to the osmotic load of retained urea

In this paper we report two cases of enormous postobstructive polyuria and natriuresis The purpose is to draw attention to this intriguing disturbance in renal function because kowledge of this type of polyuria is of crucial therapeutic importance In our cases the data suggest a proximal tubular defect as described by Bricker et al

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Case reports

Case 1 (R H Med Dept P division of nephrology, record no 278) A previously healthy 79 year old man had experienced intermittent haematuria throughout the last eighteen months He was admitted with total anuria of one day's duration after a period of increasing haematuria and frequent micturition On admission his general condition was relatively good and hydration appeared normal without oedema Haemoglobin was 10 7 g per 100 ml serum creatinine 17 4 mg per 100 ml serum urea 284 mg per 100 ml serum sodium 137 mEq per litre serum potassium 6 4 mEq per litre serum chloride 95 mEq per litre and standard bicarbonate 18 5 mEq per litre

Cystoscopy revealed a large tumour occupying most of the right side of the bladder and occluding the right ureter At the left ureteral orifice a small papillomatous tumour was observed A nephrostomy on the left side was promptly followed by brisk diuresis In the first 16 hours following operation 6 400 ml of urine was delivered through the nephrostomy catheter During this period the patient received only 2 400 ml of fluid intravenously and he became clinically dehydrated There was no glycosuria

During the first 4 days following nephrostomy the urinary output was above 10 litres per 24 hours with sodium losses of

few lumps of material passing with the urine accompanied by frequency of micturition Six months later he was admitted to another hospital complaining of urinary frequency and a bladder tumour was diagnosed An intravenous urography taken on April 24 showed a slight dilatation of the left pelvis on the right side there was no excretion of the contrast medium On May 11 an excretory urogram revealed progression of the dilatation of the left pelvis and the ureter was dilatated the right side was not visualised

On July 3 1962 the patient was admitted to the Surgical Department C Rigshospitalet He complained of lassitude and during the week before admission of occasional vomiting The state of nutrition was normal but he *appeared slightly dehydrated Cystoscopy* revealed a large bladder tumour which totally occluded the right and partially the left ureteral orifice The haemoglobin was 11 0 g per 100 ml serum protein 8 0 g per 100 ml serum creatinine 6 2 mg per 100 ml serum sodium 135 mEq per litre serum potassium 5 1 mEq per litre serum chloride 100 mEq per litre and standard bicarbonate 15 1 mEq per litre

During the next week the patient was treated with parenteral fluid and blood transfusions The urinary output was 1 010—1 420 ml per 24 hours the specific gravity 1 005—1 010 During this week the serum creatinine increased to 10 6 mg per 100 ml and the serum urea was 253 mg per 100 ml As retrograde ureteral catheterization was impossible a left nephrostomy was performed on July 11 at 6 p m

At operation the left kidney was found to be nearly twice as large as normal the ureter was as thick as a thumb Immediately following drainage profuse diuresis took place At 7 a m the urinary output amounted to 5 800 ml with a sodium excretion of 574 mEq As the polyuria continued the patient was transferred to the division of nephrology at 3 p m At the time of arrival the patient had received a surplus of 2 500 ml water and 560 mEq sodium Further data appear from table II and fig 2

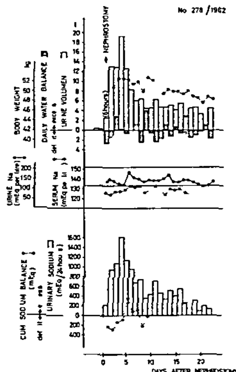


Fig 1 Case 1 Body weight water and sodium balance during the first 22 days after nephrostomy

At the time of transfer the patient was somewhat feeble but otherwise looked well The tongue was dry the skin turgor normal and there were no pulmonary rales Serum sodium was 149 mEq per litre The urine contained a trace of protein but no sugar In order to exclude the possibility that polyuria and natriuresis were partly due to overdosage of water and salt the fluid supply was discontinued from 6 to 8 p m During these two hours the urine volumes were 480 and 400 ml per hour This loss was not replaced and the sodium administration was reduced In this 24 hour period from 7 to 7 a m) the patient was in negative fluid and sodium balance Hereafter the serum sodium level was kept at a low normal and therapy directed toward replacement of current losses

On the third day after nephrostomy the fluid supply lagged behind owing to dif

TABLE II Case 2 Blood chemistry and urine investigations during the first 5 days of the stay in the division of nephrology The nephrostomy was performed at 6 p m on July 11th (see text)

	Date				
	12 7	13 7	14 7	15 7	16 7
Urine volume (ml per 24 hr)	13 540	13 100	10 480	11 500	7 260
Specific gravity	–	1 005	1 008	1 006	1 010
Serum values (8 a m)					
Sodium (mEq per l)	140	139	138	138	136
Potassium (mEq per l)	4 6	4 0	3 9	4 1	3 9
Chlorides (mEq per l)	108	105	112	111	105
Standard bicarbonate (mEq per l)	18 2	18 0	15 6	16 8	17 9
Urea (mg per 100 ml)	217	153	96	75	60
Creatinine (mg per 100 ml)	9 3	7 4	4 1	3 0	2 5
Urinary excretion					
Sodium (mEq per 24 hr)	1 380	1 350	1 040	1 070	680
Potassium (mEq per 24 hr)	190	236	273	190	152
Netto acid (mEq per 24 hr)	0	79	136	89	116
Urea (mMol per 24 hr)	946	784	647	596	448
Creatinine U/P ratio[2]	2 9	4 6	5 9	8 1	8 7
Endogenous creatinine clearance (ml per min)[1]	27	42	43	63	44
% of filtered sodium load excreted[2]	24 9	16 2	12 2	8 7	7 8
% of filtered potassium load excreted[2]	114	105	107	54	52

[1] and [2] as in table I

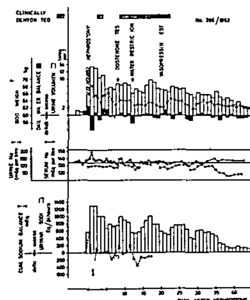


Fig 2 Case 2 Body weight water and sodium balance during the first 44 days after nephrostomy

ficulties with an intravenous drip On this day the patient lost 1 500 grams in weight and the next morning appeared dehydrated In spite of this output was 10 480 ml On the seventh day the fluid balance control failed The patient lost 1 400 g The following day the serum sodium was below normal (133 and 130 mEq per litre at 8 a m and 4 p m) In spite of this the sodium content in the urine was 86 mEq per litre

Eight days after nephrostomy, a six hour aldosterone test with injection of 1 mg aldosterone was performed Neither the hourly output nor the sodium and potassium excretion was influenced Nineteen days

after nephrostomy 2 6 and 10 i u vasopressin were injected at hourly intervals After the last injection the patient felt unwell and had a bowel movement The hourly urinary outputs remained unaltered

During the first two weeks the patient had no appetite and was fed by a stomach tube The oral sodium intake in this period was estimated from standard tables

The period of polyuria and defective renal sodium conservation lasted for 34 days following which a sudden improvement occured

On August 26 the patient was transferred back to the surgical department in excellent condition The serum creatinine was slightly elevated (1 6 mEq per 100 ml) and the endogenous creatinine clearance was 55 ml per minute

Discussion

After relief of urinary tract obstruction a copious diuresis may ensue due either to elimination of excess water retained during the period of obstruction or to an osmotic diuresis caused by excretion of accumulated urea

Both these possible causes can be excluded in our patients since neither of them were overhydrated prior to the relief of urinary obstruction and since polyuria continued after normalisation of serum urea

Overadministration of fluid during subsequent treatment can also be excluded as attempts to reduce the administration of water and salt did not cause reduction in the polyuria and natriuresis but resulted in dehydration and hyponatraemia

During partial urinary tract obstruction a massive water losing nephropathy unresponsive to vasopressin may occur (e g Roussak and Oleesky (7), Mees (4)) This disturbance of renal function must be distinguished from the abnormality dealt with here Although osmolality determinations were not performed it is evident that the polyuria in our patient was not due to lack of antidiuretic hormone or to nephrogenic diabetes insipidus This appears both from the copious natriuresis and from a simple calculation of the amount of solutes excreted, which shows that the osmolality of the urine was close to that of serum In case 2 vasopressin did not reduce the urine flow

The most conspicious feature of the polyuria in our patients was the enormous urinary sodium losses During the first three days both patients excreted more than the normal exchangeable body sodium The urinary concentration of sodium was large and relatively fixed, even in the presence of low normal or decreased serum sodium concentration This can only be explained on the basis of depression of the tubular reabsorption of sodium which in turn must have been due to a primary renal defect since evidence of adreno-cortical dysfunction was absent, and since the administration of aldosterone did not reduce sodium excretion (case 2)

A negative balance of water and sodium following urinary decompression seems to be common although usually occuring only to a moderate degree Thus moderate sodium losses and polyuria were seen in 24 cases studied by Eiseman et al (2) and in 6 patients studied by Persky et al (6) In one of Eiseman et al s patients saltloss persisted for 3 months

TABLE III Details of 11 cases of pronounced polyuria and natriuresis occurring after surgical relief

Reference	Case no	Sex	Age (yrs)	Diagnosis	Clinical comments	Duration of anuria
Wilson et al (8)	1	♂	65	Chronic urinary retention	Self catheterization previously used frequently	3 days
	2	♂	74	Prostatic enlargement	Comatose and febrile on admission	?
	3	♂	51	?	Passed urine through perineal fistulas 22 years Increased urinary frequency one week	?
Parsons (5)	1	♂	54	Prostasic enlargement	Uraemic symptoms admitted to medical dept Bladder to the umbilicus No difficulty in micturition	No anuria
Bricker et al (1)	1	♀	60	Ureter stone	Previous left nephrectomy History of right renal calculi Uraemic symptoms 3 weeks	4 days
	2	♂	76	Prostatic enlargement	Prostatic symptoms for several years increased 3 weeks prior to admission On admission somnolent	?
	3	♂	76	Occluded bladder catheter	Amputation of right leg Initial NPN normal	48 hours
	4	♂	55	Prostatic enlargement	Prolonged history of prostatic symptoms Uraemic symptoms 2¹/₂ months	?
Witte et al (9)	—	♂	52	Carcinoma of the prostate	Prostatic symptoms for 7 months aggravated the last 3 weeks	No anuria
Present cases	1	♂	79	Carcinoma of the bladder	Intermittent haematuria for 1¹/₂ years	1 day
	2	♂	62	Carcinoma of the bladder	Uraemic symptoms haematuria	No anuria

Pronounced postobstructive sodium and water losses are much rarer Details of 11 cases (including the present two) are presented in table III

The reason why a severe sodium and water diuresis only appears in a small percentage of patients is unknown Table III shows that chronic obstruction

of urinary tract obstruction

Blood urea (BU) or blood urea nitrogen (BUN) at relief (mg per 100 ml)	State of hydration at relief	Urine volume and sodium excretion				Duration of polyuria
		Period	Litres	Total (mEq)	mEq per l	
BU 310	Slightly oedematous	12 hr	8	—	—	5 days
BU 285	Skin inelastic	21 hr	7 8	—	—	2 days
BU 171	Slightly oedematous	19 hr 2 day	12 45 15	>940 c 1540	>76 c 103	c 1 week
BUN c 135	Normal	1 day 2 day 3 day	4 27 4 19 4 62	— — —	82 5 60 9 55 6	c 2 weeks
BUN 232	Mild congestive heart failure	12 hr 2 day 3 day	7 5 13 9 5	700 1140 800	93 88 84	12 days
BUN 177	Dehydrated	8 hr 2 day 3 day	3 1 7 4 9 4	— 274 350	— 37 37	Fell slowly (normal 2 months later)
—	—	24 hr output during 3½ months	8 6—15	900—1900	105—127	3½ months
BUN 58	Moderate peripheral oedema	Max 24 hr output	4 5	—	—	2 weeks
BUN 245	Normal	2 day	Max 69 ml per min	—	c 100	4 days
BU 284	Normal	3 day	19 280	Max 1620	c 100	3 weeks
BU 217	Normal	1 day	13 540	Max 1380	100—120	34 days

appears to be a salient feature however case 3 of Bricker et al (1) had only 48 hours obstruction Although the degree of obstruction always seems to be great, total anuria need not be present In our case 2 the 24 hour output was more than 1 000 ml prior to operation but a rapid deterioration in renal function had

taken place and a pronounced hydro nephrosis had developed

Studies performed by Bricker et al (1) and later by Witte et al (9) demonstrate that the condition is due to a defect in the tubular reabsorption of sodium and indicates that the defect is primarily located in the proximal tubules Detailed studies of renal function were not performed in our patients but the large excretion fraction for sodium indicates that a proximal tubular defect must also have been present in our cases The large excretion of potassium ex ceeding the filtered load (case 2) indicates that distal sodium reabsorption must have occured

The therapy of postobstructive sodium losing nephropathy consists of a pains taking replacement of the abnormal losses of water and electrolytes to prevent fatal dehydration which may develop *within 24 hours in pronounced cases* On the other hand overadministration of water and sodium should also be avoided During such circumstances the magnitude of the polyuria can become enormous Witte et al (9) observed a peak flow of 69 ml per minute (ap proximately 100 litres per 24 hours) in a patient who received abundant salt and glucose containing fluids

Summary

A report is given of two patients in whom enormous reversible polyuria and natriuresis appeared immediately fol lowing relief of urinary tract obstruction by nephrostomy

The investigations lend support to the assumption that the principal defect is a suppression of proximal tubular sodium reabsorption

Awareness of this complication is of clinical importance as it may result in rapid extracellular dehydration collapse and death

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From the First Medical Department Sahlgrenska sjukhuset University of Goteborg Gote borg and the Medical Department of the Hospital in Molndal Molndal Sweden

Blood Pressure, Triglycerides and Age in Relation to Coronary Symptoms in Hypercholesterolemia

By

G ÖRNDAHL, H SANNE, G WELIN, MARIANNE AHLSTRÖM and B HOOD

There have been many investigations of blood pressure and serum cholesterol in different countries, different races, different professions and in populations with different dietary and other habits such as smoking (4 5 9 12, 13, 14, 15) We have, however failed to find an analysis of blood pressure in hypercholesterolemia

In an earlier paper (6) we have discussed the long term prognosis as well as the effect of attempting strict dietary control in a material comprising 458 cases of hypercholesterolemia The principles for selection of this material were given in this previous paper (6) We required that two separate determinations should show serum cholesterol above 300 mg % or that definite tendinous xanthomatosis should be present

We have, in the present paper concentrated our attention to the following problems 1 Do patients with hyper cholesterolemia exhibit normal or elevated blood pressure levels? 2 Are there within the same individual correlations between the levels of serum cholesterol or triglycerides expressed as glyceride glycerol and that of blood pressure? 3 What are the relations between blood pressure cholesterol glyceride glycerol, age and coronary symptoms?

Clinical material

The patients had been admitted to the First Medical Department Sahlgrenska sjuk huset 1947—1958 or to the Medical Department of Molndal 1954—1958 During the years covered the main indications for determination of serum cholesterol were signs of ischemic heart disease or xanthomatosis The asymptomatic group included a small proportion of patients investigated due to the findings of hypercholesterolemia in relatives Serum triglyceride determination was not available until 1959 but was used at the follow up examination of the present material

The material has been subdivided into groups according to the state at the initial examinations as follows

Group I *Asymptomatic* No clinical symptoms from the cardiovascular system

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TABLE I The material at the initial examination

	Group I		Group II		Group III	
	Asymptomatic		Angina pectoris		One or more myocardial infarctions either fresh or old[1]	
	♀	♂	♀	♂	♀	♂
Total number	67	31	94	55	71	106
Age[2]	52	49	58	54	61	56
Cholesterol[2]	364	345	365	364	357	338
Systolic blood pressure[2]	165	150	180	156	176	153
Diastolic blood pressure[2]	97	94	102	96	100	95
Patients with antihypertensive treatment[3]	4	1	8	1	4	2

[1] Out of these 177 patients about 15 per cent had two or more myocardial infarctions and 68 per cent a fresh one

[2] Mean values

[3] Drugs used Reserpine Pentholinium Hydralazine Chlorothiazide

TABLE II The material at the follow up examination

	Group I		Group II		Group III	
	Asymptomatic		Angina pectoris		One or more myocardial infarctions[1]	
	♀	♂	♀	♂	♀	o
Total number	36	14	64	39	48	51
Age[2]	55	51	62	60	65	62
Cholesterol[2]	360	297	328	313	334	299
Range	264—589	225—441	216—481	173—457	237—592	187—419
Glyceride glycerol[2]	1 27	1 29	1 53	1 86	2 05	1 38
Range	0 41—2 68	0 59—2 26	0 59—6 75	0 62—6 72	0 71—13 20	0 54—3 95
Systolic blood pressure[2]	158	138	179	147	183	158
Range	110—250	110 210	110—290	105—190	130—265	110—225
Diastolic blood pressure[2]	100	92	103	92	103	98
Range	80—130	75—120	75—160	60—140	80—130	60—145

[1] Out of these 99 patients 25 per cent had two or more myocardial infarctions

[2] Mean values

TABLE III The material subdivided as to whether having strict diet or not and antihypertensive drugs or not

	Group I				Group II				Group III			
	Asymptomatic				Angina pectoris				One or more myocardial infarctions			
	♀		♂		♀		♂		♀		♂	
	Diet	No diet	Diet	No diet	Diet	No diet	Diet	No diet	Diet	No diet	Diet	No diet
Cholesterol	343	369	303	293	320	334	309	317	342	330	302	297
Glyceride glycerol[1]	1 17	1 32	1 30	1 29	1 60	1 48	1 47	2 16	1 76	2 24	1 42	1 36
No of patients	13	23	5	8	27	37	17	22	18	28	20	31
	Drugs	No drugs	Drugs	No drugs	Drugs	No drugs	Drugs	No drugs	Drugs	No drugs	Drugs	No drugs
Diastolic blood pressure	112	98	—	92	109	101	113	90	111	98	90	99
Number of patients	5	30	—	14	18	46	4	34	19	29	4	45

[1] Mean values

Group II Angina pectoris In a few patients there was co-existent intermittent claudication

Group III One or more myocardial infarctions either fresh or old Symptoms as in group II may or may not be present

These three groups have been divided according to sex Data are given in table I

Group II Mixed group To this group we have referred cases with intermittent claudication arteriographically proven occlusion of leg vessels and cerebro-vascular lesions This group contained 17 females and 17 males This mixed group has been taken into account only in the survey of the material at the initial examination and in the elucidation of our first problem do patients with essential hypercholesterolemia exhibit elevated blood pressure?

As many as possible of the survivors have been examined between 5—16 years after the initial examination The clinical examination included palpation for xanthomatosis and sphygmomanometric recording of blood pressure in both arms in the horizontal position after at least 15 minutes in the horizontal position and after two minutes standing Diastolic pressures were read at the disappearance of the sounds Clinical histories have been reviewed with special regard to agents that lower blood pressure or lipids as well as to dietary habits ascertained by questioning The term strict dietary regimen was defined in a previous paper (6 No patient had been dieting before the inital examination Sustained treatment with anticoagulants had been given in only a few cases Cholesterol and triglyceride levels were determined in the fasting state at the follow

Fig 1 Blood pressure of females without symptoms (○) and with angina pectoris (●) at the initial examination are plotted in Humerfelt s diagram from Bergen Systolic and diastol c pressure in the left and right part respectively

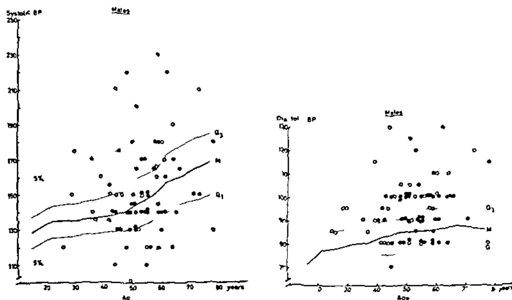


Fig 2 Males Explanations as in fig 1

up examination The chemical methods have been described in a previous paper (6)

The same grouping has been used at the follow up as at the initial examination If an individual had developed angina pectoris or a myocardial infarction in the meantime he has been transferred to the appropriate group

Results

Some data on the material at the initial examination have been given in table I

Table II shows some of the results at the follow up examination In table III the material has been further subdivided as to whether or not they had strict diet or whether or not they had received active antihypertensive agents

To discuss the *first problem*, i e what were the blood pressure levels in hyper cholesterolemia, we have done the below analysis As we have no large population studies in Goteborg on blood pressure levels covering all age groups in the present material we have used as a background for our own data the excellent data of Humerfelt from Bergen (8) Geographically not far apart, Bergen and Goteborg show a number of similarities

We have obtained an age related comparison with the Bergen figures by plotting our data for blood pressure against Humerfelt s diagrams showing the median and quartile lines (and also showing for the systolic pressure, the lines above and below which 5 per cent of the Bergen population fell) We have thus plotted 8 diagrams, separating the two sexes and also separating those who had had a myo cardial infarction from those who had not (combined asymptomatic and an gina pectoris groups) We have also plotted the blood pressures obtained at the initial examination in separate diagrams from those obtained at the follow up examination Two examples of this have been given in fig 1 and fig 2

TABLE IV Group IV or mixed group divided according to blood pressure Number of patients in the quartiles of the Humerfelt s diagrams

Q_4 = the highest quartile highest pressure

	Intermittent claudication		Cerebro-vascular lesion	
	♀	♂	♀	♂
Systolic blood pressure				
Q 4	2	3	6	3
Q 3	2	3	1	1
Q 2	2	2	2	1
Q 1	1	3	1	1
Diastolic blood pressure				
Q 4	5	4	7	4
Q 3	1	6	1	–
Q 2	–	1	1	–
Q 1	1	–	1	2

The percentage of the series falling above and below the median and quartile lines as well as (for the systolic pressure) above or below the five per cent lines have been given in table V In this table we have also given the percentage figures for those who in the various group of the material exhibited diastolic pressures of 100 mm Hg or more

From table V it is seen that at the follow up examination there was a high

TABLE V Distribution of blood pressures in the present material of hypercholesterolemia against the background given by the Bergen population study

	The initial examination				The follow up examination			
	♀		♂		♀		♂	
	Groups I+II	Group III	Groups I+II	Group III	Groups I+II	Group III	Groups I+II	Group III
Total number	161	71	86	106	100	48	53	51
Systolic blood pressure above the 5 % line	20	22	10	11	12	12	2	6
Above the top quartile line	46	42	35	32	37	40	19	31
Above the median line	69	62	57	50	60	74	32	49
Below the median line	31	38	43	50	39	26	68	51
Below the low quartile line	14	24	18	30	18	16	32	31
Below the 5 % line	4	8	5	12	4	4	6	6
Diastolic blood pressure above the top quartile line	57	58	57	53	60	66	37	58
Above the median line	79	70	77	72	88	86	70	80
Below the median line	21	30	23	28	11	14	30	20
Below the low quartile line	10	19	2	11	4	4	8	6
Above the line of 100 mm Hg	54	57	43	43	51	66	28	55

Group I = asymptomatic
Group II = angina pectoris
Group III = one or more myocardial infarctions

frequency of elevated blood pressure in all groups (particularly among the females) with the exception of the male group I and II (combined asymptomatic + angina pectoris) In this group only 32 per cent showed systolic pressures above the median line and of these 19 per cent fell in the highest quartile

The frequency of elevated diastolic levels was higher than for elevated systolic levels The percentage of cases with diastolic pressures above the median line was between 70 and 85 per cent, while the corresponding figures for systolic pressures were between 32 and 73 per cent At the follow up examination more than 50 per cent had diastolic pressure in the highest quartile with the exception of the male groups I and II (asymptomatic + angina pectoris) The percentage of systolic pressures falling in the upper quartile varied between 19 and 46

As seen from figs 1 and 2 and from table V the frequency of elevated pressures was somewhat higher in the females than in the males

The frequency of elevated pressures was somewhat higher in both males and females at the initial examination in the groups without than in those with myocardial infarction However, 68 per

cent of the myocardial infarctions were fresh ones At the follow up examination the conditions were reversed

The frequency of those having diastolic pressures above 100 mm Hg was higher in the females (55—64 per cent) than in the males (30—59 per cent) At the follow up examination the frequency of diastolic pressures of 100 mg Hg or more was higher (59—64 per cent) in those with myocardial infarction than in those without myocardial infarction (30—50 per cent)

Details of the heterogeneous group IV (intermittent claudication, cerebro vascular lesion) are given in table IV Likewise in this group there was an increased frequency of elevated pressures more often in the females

The second problem put forward i e what were the intra individual relations between lipid parameters and blood pressure was treated as follows Plotting of serum cholesterol and triglyceride separately against systolic as well as diastolic blood pressure in the same individual, in groups without and with myocardial infarction, revealed not the slightest tendency to correlation Thus we have found no signs of correlation between individual lipid and blood pressure levels in this material of hyper cholesterolemia

The third problem, the relationships between age blood pressure serum cholesterol or serum glyceride glycerol and the absence or presence of coronary symptoms, is analyzed in table II This table gives the average figures for all cases submitted to the follow up examination Average age and average glyceride glycerol were higher in the groups with symptoms than in the asymptomatic ones No such tendency was evident for average blood pressure or serum cholesterol

The results shown in table I as regards serum cholesterol and blood pressure levels at the initial examination did not show consistent differences between the asymptomatic, the angina pectoris and the myocardial infarction groups In the following figures (3, 4, and 5) the results at the follow up investigation have been sub-divided according to sex and decade With the single exception of females 50 years or below with myocardial infarction, in whom the average serum cholesterol level was very high no systematic differences in average serum cholesterol were found in any of the sub groups In the two younger groups of males with vascular symptoms there were on the average somewhat higher diastolic blood pressure levels than in the groups without symptoms

Glyceride glycerol was however higher in 13 of the 14 groups with symptoms than in the corresponding asymptomatic ones In the group of males above 70 years there were no asymptomatic cases

An interesting sex difference was observed For the males in all decades average glyceride glycerol was higher in the group with angina pectoris than in those with myocardial infarction where as the females with myocardial infarction in the decade 51—60 as well as 61—70 had a considerably higher average glyceride glycerol than the other groups and definitely higher than those in the same age and sex groups with angina pectoris As several of these

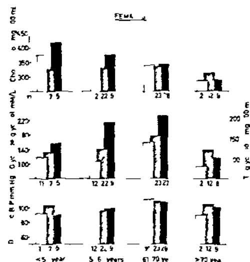


Fig 3 Females The mean values in four age groups for cholesterol glyceride glycerol and diastolic blood pressure
□ = asymptomatic
□ = angina pectoris
■ — one or more myocardial infarctions

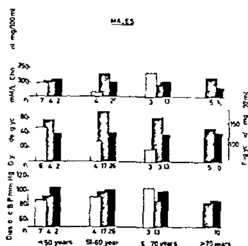


Fig 4 Males Explanations as in fig 3

groups were rather small and as there may be extraordinary variation particularly in glyceride glycerol values, the trends in the average figures might have been due to inclusion of a few individuals with extremely high values To test this we have plotted individual glyceride-glycerol values in males and females between 61—70 (fig 5) As seen from this figure the explanation for the difference between the two sexes and between groups with and without symptoms does not lie in the inclusion of stray individuals with extreme values The same conclusion holds for the other age groups

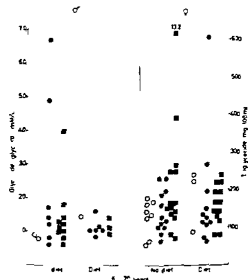


Fig 5 Individual glyceride-glycerol levels (on the abscissa to the right converted to triglycerides in both sexes in the age group 61—70 years of age Material subdivided into those on strict diet and those without
○ = asymptomatic
● = angina pectoris
■ one or more myocardial infarctions

Discussion

Both the groups with and those without coronary symptoms showed a high frequency of cases with elevated blood pressures In asymptomatic hypercholesterolemia particularly, this seems of definite interest There was, however,

a difference in methods used, the blood pressures in the Bergen series being measured in the sitting position by a nurse In the present series they were measured in the horizontal and standing positions by a physician The comparability of our hospital material and the general population study of Bergen can be questioned With these reservations the comparison seems however, to give an idea of the distribution of pressure in different age and symptom groups

The fact that about half of our series had diastolic levels of 100 mm Hg or more serves also to demonstrate the increased frequency of hypertensives One possible explanation for the high incidence of elevated pressures in asymptomatic hypercholesterolemia might be as follows The patients acquiring coronary symptoms at an early age did so not only due to their hypercholesterolemia but also in many cases due to an increased blood pressure level To the extent that the asymptomatic patients in our series were siblings deliberately called for examination after vascular disease had been found at an early age in their relatives the same increased incidence of hypertension might well have been encountered Not more than one third of the asymptomatic individuals were obtained in this way and in this group there was a low incidence of elevated pressures

Work of our own (Hood et al (7)) starting out from patients with renal artery stenosis, has shown a significantly higher average glyceride glycerol and a somewhat higher serum-cholesterol level in this group than in control normotensives and other hypertensives There was also a significantly higher number of individuals showing clearly elevated values of glyceride glycerol In the present series only few subjects have been submitted to renal arteriography It is thus not possible to know how often the elevated blood pressure in hypercholesterolemic and/or hyperglyceridemic subjects may be explained by the presence of renal artery stenosis

There have been some earlier investigations about the relationship between serum cholesterol and blood pressure in healthy subjects and in subjects with hypertensive disease Malmros et al (10) found no correlation between total serum cholesterol and systolic blood pressure in healthy Swedish males in age groups 20—29 and 50—59 years Mathur et al (11) who studied 78 cases with essential hypertension found serum cholesterol levels to be higher in the hypertensives than in healthy persons They could give no explanation for this finding Waris (15) found however no significant correlation between serum cholesterol levels in 101 patients with hypertension and 60 healthy controls In the present series there was no correlation between each of the lipid parameters and the blood pressure in the individual patients

The analysis of the survivors in this material follwed for between 5—16 years and originally selected on the basis of an elevated level of cholesterol and/or tendinous xanthomata showed no consistent difference in average diastolic blood pressure or average serum cholesterol in groups with or without vascular symptoms One exception was that there were somewhat

higher diastolic levels in the male groups with coronary symptoms in the decades 50 or below and 51—60 There was also the exception of high average serum cholesterol in females 50 or below with myocardial infarction The higher average glyceride glycerol in "coronary" than in asymptomatic groups was striking, as was the contrast between the two sexes when the angina pectoris groups were compared with myocardial infarction groups The latter finding might be due to the earlier rise of glyceride glycerol in the male as age advances (2), which may be one of the important factors leading to an earlier appearance of coronary disease The higher average glyceride glycerol in angina pectoris as compared with myocardial infarction might mean an elimination of many of those with high triglycerides through lethal myocardial infarctions

The salient interesting fact remains that even in hypercholesterolemic individuals the level of glyceride glycerol seems to play a great role in determining whether or not symptoms will be present in the various age groups In most of the groups with coronary symptoms the mean glyceride glycerol values were considerably higher as compared with corresponding age groups from controls investigated in the same laboratory (1)

The caution necessary in interpretation is obvious All the data lost with those who died during the long observation time coupled with the lack of glyceride glycerol determinations at the initial examination are disturbing factors There are other factors distorting the pattern at the follow up examination A few members of the families with the most advanced xanthomatosis and the most ominous family histories frankly refused to be reminded more than necessary of their condition and have refused to come to the follow up examination Secondly, there is the distortion of lipid levels and blood pressure produced by diet or antihypertensive treatment However, the adherence to a strict diet was somewhat more frequent in groups with coronary symptoms than in those without If the assumption is made that diet had lowered the glyceride glycerol, one might suppose that this should affect mean values somewhat more in the groups with coronary symptoms than in the asymptomatics It would thus have been reasonable to expect differences greater, not smaller, than those actually found There was also a somewhat higher incidence of cases treated with active antihypertensive treatment at the follow up examination in the groups with coronay symptoms However, the incidence was not more than one third of all patients and in the majority of the cases only thiazide was used Even if one assumes a significant decrease of blood pressure of the magnitude usually produced by thiazide in one third of the population, this would only to a small degree affect the average diastolic blood pressure levels in the groups taken as a whole We have approximately calculated this to be of the order of 5 mm Hg Of relevance is the fact that results at the initial examination showed no significant differences in diastolic blood pressure level between those with and those without coronary symptom

Summary and conclusions

The present material of 458 patients, originally selected on the basis of elevated serum cholesterol and/or tendinous xanthomata and followed for 5—16 years, has been submitted to a follow up examination The main findings were as follows

1 Plotted against the background data given by the population survey of Bergen, diastolic blood pressures for roughly one half of the material fell in the top quartile of the Bergen data Moreover in approximately half of the material diastolic pressures were 100 mm Hg or more Diastolic pressures were also somewhat higher in the female group This tendency was the same in all sub groups — asymptomatic cases, cases with angina pectoris, and cases who had survived one or more myocardial infarctions

2 No correlation was found in the individual patient between serum cholesterol or glyceride glycerol on the one hand and blood pressure on the other

3 At the follow up examination in age groups 50 and below 51—60 and above 70 the following findings were made a) With the exception of the high average serum cholesterol in females below 51 with myocardial infarction, no systematic difference was found in cholesterol between groups with and groups without coronary symptoms b) There was a trend to somewhat higher diastolic blood pressure levels in males in groups below 61 years with coronary symptoms than in those without c) The glyceride glycerol values were in 13 of the 14 subgroups with coronary symptoms higher than in the corresponding sex and age groups without such symptoms In males more than 70 there were no asymptomatic individuals In a few groups the average figure was approximately twice as high as in asymptomatic hypercholesterolemia In the males the angina pectoris groups showed the highest levels in the females the myocardial infarction groups The reservations inherent in the study after this long time of observation have been discussed

Acknowledgement

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From Medical Department B Copenhagen County Hospital in Gentofte Hellerup and
Medical Department B Glostrup Hospital Glostrup Denmark

The Relationship between Pregnancy and Haemorrhagic Proctocolitis

By

EVA WEEKE, VIBEKE BINDER, J H OLSEN POVL RIIS and POUL ANTHONISEN

That haemorrhagic proctocolitis often coincides with pregnancy is a well-established fact to anyone who has some experience with the disease During the last thirty years the relationship between pregnancy and proctocolitis has been the object of several investigations Though most of these have indicated that the frequent simultaneous occurrence can hardly be explained by simple coincidence and though important clinical aspects of the problem have been elucidated, our knowledge is still incomplete concerning practical implications of the relationship and a deeper understanding of the pathogenetic mechanisms which underlie the interaction of the two conditions is still lacking

The advances of recent years with regard to methods of examination (e g bioptic and cytological techniques) have lowered the diagnostic threshold of proctocolitis and consequently made possible a collection of patient materials including a larger number of mild cases A prognostic estimation concerning both proctocolitis and pregnancy will, of course, be influenced by the composition of the patient material under study The present investigation intends to throw light on the practical problems of the proctocolitis/pregnancy combination with reference to the full clinical spectrum of the colon disease as it is seen to-day

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Material and method

The investigation comprises 94 women belonging to the group of patients with haemorrhagic proctocolitis from the Copenhagen County which is being followed by regular out patient examinations and during possible necessary admissions to the County Hospitals

As a part of the prospective collection of data from patients with proctocolitis information concerning pregnancies is recorded During the autumn of 1964 moreover questionnaires were given or sent to all female patients included in the group the dead line for replies being January 1st 1965

Satisfactory replies were obtained from 94 of the 103 patients who received a questionnaire 91 per cent) In two cases the answers

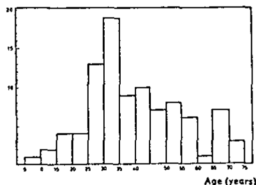


Fig 1 Age distribution on January 1st 1965 of the 94 female patients with proctocolitis comprised by the investigation

were useless because of the patients senility one patient had emigrated and could not be located, and six patients for obscure reasons did not respond to several appeals although *they were known to be alive*

The composition of the patient material with regard to age appears from fig 1 The material comprised all degrees of severity of the disease and extension of the process in the rectum and colon from mild proctitis to severe total proctocolitis The course was fulminant in two patients while 20 had the chronic continuous type of disease and 65 the chronic intermittent form of the disease Seven patients had been ill less than two years so that the course of the disease could not be classified In 20 patients colectomy had been performed Two patients died in connexion with operation for the disease neither of these had been pregnant Conditions unconnected with the colon (arthritis liver disease and/or skin eruptions) had appeared in 20 patients

Results

Twenty one patients had passed the menopause before the first attack of proctocolitis, and one had not reached the menarche at the time of this investigation *(table I, above)* The remaining 72 women had been in fertile age during the greater or lesser part of the course of their disease Fifty had been pregnant (table I, below) and had born 113 living children in all Fifteen women only had been pregnant before the first appearance of proctocolitis, of these seven were over 35 years of age at the first symptom, and in six younger women the disease had not commenced until 1960 so that the time of observation with regard to secondary sterility was too short The indicated causes of childlessness in the 22 patients who had not been pregnant although they had been in fertile age during the course of the disease are listed in table II It is noteworthy that none gave the disease as a *reason for voluntary childlessness*

Thirty five women had been pregnant during the course of proctocolitis, 13 once and 22 more than once Their total number of pregnancies was 71

The influence of pregnancy on proctocolitis Table III shows the degree of disease activity at the beginning of pregnancies In 14 women the colon disease commenced during pregnancy or puerperium (defined as six weeks post partum), the starts of the remaining pregnancies were almost equally divided between active and quiescent phases of proctocolitis 'Active phase' has been defined as occurrence of diarrhoea and/or bleeding from the rectum plus possible pain, 'quiescent phase' as the absence of these symptoms

The course of the disease during pregnancy in the two groups of activity appears from fig 2 In the 'active *phase' group the proctocolitis grew*

TABLE I Above distribution of the 94 female patients with proctocolitis with regard to temporal relationship between disease and fertile age Below temporal distribution of pregnancies for the 72 women who had been in fertile age during course of proctocolitis

	Pre fertile age	Fertile age	Post fertile age	Total
Number of patients	1	72	21	94

	Pregnant during course of disease	Pregnant before onset of disease	Not pregnant	
			Married	Not married
Number of patients	35	15	7	15

TABLE II Causes of childlessness in the 22 women who had been in fertile age during course of proctocolitis but who had not been pregnant

	Unmarried state	Voluntary because of disease	Involuntary	Not stated
Number of patients	15	0	4	3

TABLE III Disease activity at commencement of pregnancies

	Active phase	Quiescent phase	Onset during pregnancy	Onset during puerperium	Total
Number of pregnancies	27	30	11	3	71

worse in about a quarter of cases, improved in about a third, and was unaltered in the remaining In the "quiescent phase group the condition became worse in about a quarter of the cases while the disease was unaffected in the remaining

In the 11 patients with onset of the disease during pregnancy the first symptoms were distributed almost equally over the whole period of [illegible] with only a small prepond[illegible] first half

Of the 14 cases of pr[illegible] onset during pregnancy [illegible] 11 were mild to mod[illegible] only three severe

Table IV shows [illegible] colitis during succe[illegible] the 22 multiparae [illegible]

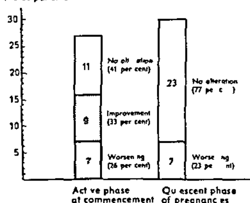


Fig 2 The influence of pregnancies on the course of proctocolitis

pregnancies comprised in the present investigation (included are those pregnancies during which or during the puerperium of which the disease commenced) The course of the disease was differently affected from one pregnancy to another in about 2/3 this is also true of the eight multiparae in whom the proctocolitis had first appeared during pregnancy or puerperium

As it has been alleged that the attitude of the patient towards the expected child affects the course of the disease during pregnancy (5) we have tried to clarify this point in the present investigation Naturally the patients were not always able to give very exact information concerning their mental states during past pregnancies, so that the answers must be evaluated with caution (table V) There were no unwanted children in the group of patients who improved during pregnancy, but beyond this the investigation does not support the above mentioned assertion

The influence of proctocolitis on pregnancy The relation between the results of pregnancies and the course of proctocoli

TABLE IV Influence of proctocolitis on successive pregnancies in the 22 multiparous women

	Deterioration during all	Improvement during all	Unaltered during all	Dissimilar influence
Number of patients	2	0	6	14
Per cent	9	0	27	64

TABLE V Influence of pregnancy on proctocolitis in relation to patients att tude towards expected children

	Onset during pregnancy or puerperium	Deterioration during pregnancy	Improvement during pregnancy	No alteration during pregnancy
Child wanted	2	8	5	7
Child not wanted	4	2	0	7
Indifferent feeling	8	4	4	20

TABLE VI Relationship between disease activity during pregnancies and their results

	Onset during pregnancy or puerperium	Deterioration during pregnancy	Improvement during pregnancy	No alteration during pregnancy	Total
Normal deliveries	12	6	8	25	51 (72%)
Pathological deliveries	[1]2	[2]4	0	[3]2	8 (11%)
Spontaneous abortions	0	1	1	3	5 (7%)
Legally provoked abortions	0	3	0	4	7 (10%)

[1] One still birth one premature delivery
[2] Two still births one premature delivery one delivery of live malformed child (hydrocephalus)
[3] One still birth one premature delivery (provoked because of Rhesus immunization)

tis appears from table VI The groups 'Improvement during pregnancy' and 'Onset during pregnancy or puerperium' show the lowest figures with regard to pathological deliveries and abortions In spite of the rather small numbers a certain importance can probably also be attached to the fact that the total of pathological deliveries and abortions equalled the number of normal deliveries in the group 'Deterioration during pregnancy' It must be remembered however, that the former figure includes legal abortions carried out for medical reasons during quiescent as well as active phases of the disease

Discussion

A survey of previous publications on the relationship between pregnancy and haemorrhagic proctocolitis was given in 1958 by Bacon (1) On the basis of these smaller series and his own experience Bacon tried to calculate figures for the chances of improvement and deterioration of the disease during pregnancy The different materials are, however, not comparable, and the calculations are obscure

Bacon's survey did not include two larger materials from 1956 i e MacDougall s (4) and Crohn et al s (2) The former comprised 100 pregnancies in 64 women In 26 the disease commenced during pregnancy or puerperium, this group contained many severe cases Inactive proctocolitis was hardly ever affected by pregnancy (in the present material in a quarter of cases) active disease grew worse in a quarter (same fraction as in the present series) improved in a quarter and was unaffected in half During successive pregnancies the colonic disease was differently affected in accordance with our experience No influence of proctocolitis on pregnancy could be demonstrated

Crohn et al s investigations concerned 150 pregnancies in 110 patients During 74 pregnancies in 47 women with in active proctocolitis deterioration occurred in 54 per cent (i e in twice as many as in the present investigation) during

38 pregnancies in 25 women with active disease this grew worse in 75 per cent (our figure 25 per cent), most often in the first trimester In the 19 patients whose disease had commenced during pregnancy the proctocolitis was severe in 13 In the other 19 cases which appeared within six months after delivery the disease was mild in six, severe in seven, and information on the remaining cases was lacking

The present investigation has not confirmed the finding of MacDougall and Crohn et al that proctocolitis commencing during pregnancy or puerperium tends to run a particularly severe course On the contrary, in our series mild and moderately severe cases dominated this group This discrepancy between our investigations and in particular those of Crohn et al is hard to explain but is probably caused by the different compositions of the patient materials

Recently de Dombal et al (3) have published the results of a retrospective study based on a follow up of a series of women with proctocolitis during pregnancy and the subsequent three months The investigation together with compiled data from the relevant literature showed a risk of exacerbation of disease during this time of 44 per cent, as compared with a risk of 47 per cent per patient year' in a control group of fertile but not pregnant, women On these grounds the authors claimed that pregnancy has no influence on the course of proctocolitis The approach of de Dombal et al s study is exemplary, but certain features cast doubt on the validity of the conclusions Firstly the authors own material consisted almost exclusively of cases *of proctocolitis that were inactive* at the beginning of pregnancy, which must be the reason why they included data from the literature in their evaluation Secondly the comparability of the control material is not well documented, e g information concerning the composition of the material with regard to disease activity is lacking

The ideal way of solving the problem would be to compare the relapse rate of the disease during pregnancy with the relapse rate during previous and subsequent non pregnant years in the same women With a capricious disease as haemorrhagic proctocolitis the determination of average relapse rates per year in individual patients will, however, prove difficult, if not impossible

Summary

The relationship between pregnancy and haemorrhagic proctocolitis has been investigated in 94 women

Seventy two of the patients had been in the fertile age group during some part of the course of proctocolitis Of these 50 had been pregnant, 15, however, only before the first appearance of symptoms

Thirty five women in the course of the disease underwent 71 pregnancies in all Thirteen had been pregnant once, 22 more than once

With the onset of pregnancy in an active phase of proctocolitis deterioration occurred in about a quarter, while the disease improved in about a third and was unaffected in the remaining patients With the onset of pregnancy in a quiescent phase about a quarter also

became worse, while the disease was unaffected in the remainder

The proctocolitis of 14 patients commenced during pregnancy or puerperium Only in three of these was the disease of severe type, in the remaining mild to moderately severe

In about two thirds of 54 pregnancies in multiparous women no correspondence was demonstrated of the influence on the course of proctocolitis from one pregnancy to another This also held good of the cases which commenced during pregnancy or puerperium

Psychic factors played no convincing role in the course of proctocolitis during pregnancy

For the pregnancies during which the colonic disease grew worse the total of pathological deliveries and abortions equalled the number of normal deliveries This is probably a consequence of the detrimental influence of exacerbations of proctocolitis on pregnancy

Acknowledgement

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From the Roentgen Diagnostic Department and the Department of Medicine University of Goteborg and the Roentgen Diagnostic Services I and III and the First Medical Service Sahlgrenska sjukhuset Goteborg Sweden

Neurogenic Disorders of the Bladder in Diabetes Mellitus

A Clinical roentgenological Investigation

By

O Bartley, Inger Brolin, S E Fagerberg and L Wilhelmsen

Marchal de Calvi (2) is considered as being the first to describe neurogenic disorders in diabetes mellitus He also assumed that these disturbances could cause dysfunction of the bladder Bladder disturbances of neurogenic type in diabetes mellitus have also been described later by several authors but as a rule the materials have been rather scanty (1 11 12, 17 19, 22, 23)

Up to 1953 86 cases had been described and these were summarized by Spring and Hymes 1953 (21) They found that the frequency of bladder disturbances with diabetes mellitus amounted to 2 % Martin 1953 (14) accounted for a material of 150 cases with diabetic neuropathy In 8 % of these cases subjective complaints pointing to neurogenic bladder disturbances were traced

The most common symptoms have been those indicating urinary retention or incontinence, sometimes they have been combined with impotence

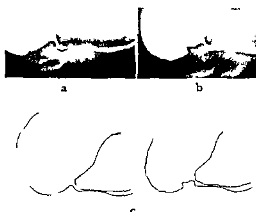
Some of the authors mentioned have studied the bladder function with cystoscopy or cystometry

The symptoms of the urinary tract are frequently uncharacteristic and figures of frequency based on the patients' subjective symptoms are thus unreliable The purpose of this work is to give an account of a systematic roentgenological investigation of the function of the bladder in an unselected diabetic material In addition the relationship between bladder disturbances and other diabetic manifestations will be discussed An investigation of this kind has never before been described

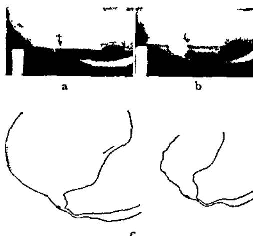
Material and methods

The study population consisted of 75 patients — 28 men and 47 women all of whom were admitted to a medical department for investigation This was carried out only at times when the patients were found to be in a good metabolic state Roentgenologically

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F g 1 Internal sph ncter d sorder I a eral v ew a) At beg nn ng of m ctu on b At end of m c ur t on Fa lure of nternal sph ncter to relax Relaxat on of tr gone c) Dra ng corre spond ng to a) and b) w th l e pos on of the bladder at rest shown by do ted l ne In connect on w h m ctur on — ex ens e lower ng of the bladder b se



F g 2 External sph ncter contrac e l bo h at start of m ctur on (a) and at end of m ctur t on (b) Max mum d lata on of n ernal sph nc er c) Drawing correspond ng to a) and b) Lateral v ews

cases of pro tat c enlargement may gi e d ffcult es n est mat on of the sph ncter funct on For th s reason all men more than 45 years of age were excluded but apart from th s exclus on there as no other selec t on of the mater al The mean age of the male pat ents as 34 1 2 years (17—45) and females 55 3 years (17—73)

The follow ng cl n cal data ere analysed the d rat on of d abetes appearance of neuropathy ret nopathy symptoms from the lower ur nary tract and bacteriur a Neuropathy was cons dered establ shed upon observat on of sensory or mo or d sturbances and or when patholog cal changes were ev dent on electromyography EMG I a gerberg et al (5) The anamnes s ncluded quest ons on frequency of m cturit on aches and pa ns on vo d ng ncont nence po er of the ur nary jet d ffculty n emptying the bladder and if in such a case extra abdom nal pressure had to be appl ed

The roentgenolog cal invest gat on of the bladder was carried out n the form of m cturit on urethrocystography accord ng to the method described by Kjellberg et al 1957 (13) Subsequent to insert on of cathe ter in the bladder th s was f lled w h a spec al barium suspens on unt l the pat ent had a feel ng of urgency F lms ere exposed prior to and dur ng urinat on w th the pat ent in a s tt ng pos t on They were taken s multaneously n two project ons frontal and lateral w th the use of an a o nat c f lm changer Us ng th s techn que the follo ng cond t ons could be stud ed sph ncter funct on s ze and shape of bladder degree of lower ng of the base of the bladder in connection with m ctur t on pos on of the nternal orifice of the urethra and presence of retent on

Normall vo d ng starts s m ltaneo sl with lo er ng of the pel ic floor and contrac t on of the detrusor muscles Th s contrac t on causes the tr gone to mo e back ards In th s way the bladder neck and the n er nal sph ncter are opened (9 13 15 A d sturbance of the internal sph ncter ex s s if the tr gone s not lowered and the sp h nc er fa ls to w den f g 1)

When the ur ne reaches the external sph ncter th s beg ns to open Its func on thus depends on an un nterrupted flow th ough the internal one

When funct onal disturbances ex ed n the lat er one the study of extern l sph nc er



Fig 3 Lateral view during micturition a) Normal shape of bladder b) Atonic bladder

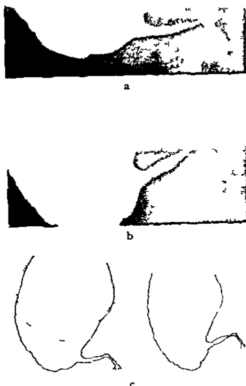


Fig 4 Ventrally located internal urethral orifice Lateral views a) At start of micturition b) At end of micturition c) Drawing Extensive lowering of bladder base during micturition

was considered impossible This can be opened and closed voluntarily, in view of the fact that it consists mainly of striated muscles (16) This also contributed to the difficulties in studying the external sphincter (fig 2)

The size of the bladder was considered normal if the patient felt urgency of voiding before a maximum volume of 500 ml of barium suspension had been injected When 600 ml or more was required the bladder was considered large In estimation the size of the bladder all cases with a volume of between 500 and 600 ml were omitted

At rest the filled bladder has a transverso-oval shape but during micturition it becomes spherical (3 9 13) If the bladder even after micturition had started retained the same shape as at rest it was characterized as atonic (fig 3)

A lowering of the base of the bladder during micturition could be studied on the lateral films The difference between the position of the bladder base at rest and maximum descent during micturition was measured directly on the films to an accuracy of 0 5 cm

The internal orifice of the urethra is normally at the lowest point of the bladder during voiding If the orifice was situated ventrally the urethra was considered as having a pathological position (fig 4) The position of the orifice was estimated on lateral projections

TABLE V Relationship between sphincter function and bladder size bladder shape and urinary retention

Sex	Sphincter function	Size of bladder		Shape of bladder		Urinary retention	
		No of cases	Frequency (%) of large bladders	No of cases	Frequency (%) of atonic bladders	No of cases	Frequency (%) of retention
♂	Normal	9	33	12	50	13	0
	Internal sphincter disorders	10	90	11	91	11	55
	External sphincter disorders	3	100	4	25	4	25
♀	Normal	19	58	20	30	22	14
	Internal sphincter disorders	6	50	6	33	6	67
	External sphincter disorders	18	44	19	21	19	37
Total	Normal	28	50	32	38	35	9
	Internal sphincter disorders	16	75	17	71	17	59
	External sphincter disorders	21	52	23	22	23	35

shape of bladder (atony) was observed in 40 % of the cases Pathologically located position of the internal urethral orifice and retention was established in approximately 25 % of all the cases

Disorders were noted in the internal as well as external sphincter These latter were of two types In some cases the sphincter was contracted during the entire investigation, in other cases repeated contractions occurred The frequency of these external sphincter disorders was equal in the study population, and both types were also equally related to other changes and were therefore grouped together Disorders of the internal sphincter in men were significantly more frequent than in women ($t = 2.50$) whereas disturbances in the external sphincter appeared to be more common in women In this respect however, the difference in sex cannot be considered as definitely ensured in view of the fact that the function of the external sphincter was not taken into consideration when internal sphincter disturbances were present These were found more often in the male patients Abnormal bladder shape was significantly more frequent in men than

TABLE VI Relationship between complications and sphincter disorders

Sex	Complications	No of cases	Frequency of sphincter disorders (%)	
			Internal	External
♂	−	15	13	20
	+	13	69	8
♀	−	15	0	53
	+	32	19	34
Total	−	30	7	37
	+	45	33	27

in women whereas the condition was the reverse concerning the localization of the internal urethral orifice In nearly a third of the women this was not situated at the lowest point of the bladder

No relationship between disorders of the external sphincter and the presence of bacteria in the urine could be established

The lowering of the bladder bottom during micturition could be determined in 22 men and 40 women The average for women was 2 3 cm and for men 1 3 The difference is significant ($t =$ 3 0) In women no relationship existed between the degree of lowering of the bladder bottom and the number of pregnancies

Table V shows the relationship between sphincter function and the size and shape of the bladder as well as urine retention It will be seen from this table that significant relationships existed in the men between disturbances in the function of the internal sphincter and both size and shape of bladder The frequency of both large and atonic bladders was greater in cases of disorders of the internal sphincter No such connection could be established among the women In both sexes urinary retention occurred significantly more often when the internal sphincter failed to function properly

In men the lowering of the bladder bottom was significantly greater when internal sphincter disturbances were present (1 9 cm) than in cases without these disorders (0 9 cm) In addition a lowering of the bladder bottom in the men was related to the occurrence of urinary retention which was established in 7 cases (2 2 cm) In 15 men without retention the corresponding figure was 0 8 cm As far as the women were concerned the tendency was similar but not statistically significant The average degree of bladder bottom descent in 25 women with normally located internal urethral orifice was 1 8 cm compared with 3 0 cm for 13 women with pathologically located urethra The difference is significant ($t =$ 2 41)

Comparison between cases with and without clinical complications The term clinical complications refers to the presence of either neuropathy in other organs or

TABLE VII Relationship between complications and size and shape of bladder

Sex	Complications	No of cases	Frequency (%) of large bladders	No of cases	Frequency (%) of atonic bladders
♂	−	11	45	15	47
	+	11	90	12	83
♀	−	13	46	14	21
	+	30	53	31	29
Total	−	24	46	29	31
	+	41	63	43	44

TABLE VIII Relationship between complications and number of roentgenological findings

Sex	Complications	No of cases	Number of roentgenological findings				
			0	1	2	3	4
♂	−	11	3	4	1	2	1
	+	11	0	1	1	7	2
♀	−	13	4	4	4	1	0
	+	29	3	13	5	6	2
Total	−	24	7	8	5	3	1
	+	40	3	14	6	13	4

retinopathy, or both The series of patients was divided into 2 groups, one group including patients with either one or both complications, and the other without either of them A comparison was then established concerning clinical data and roentgenological findings *The relationship between diabetic duration and complications has been discussed above (tables II and III)* In respect of other clinical data there was no statistical difference between the groups with an without complications

The relationship between the occurrence of complications and sphincter disturbances has been shown in table VI It was found that an intimate relationship existed between complications and disturbances of the internal sphincter Disorders of the latter type were found in 11 men and 6 women In all the female cases, as well as in 9 of the males, complications existed It was not possible to trace a relationship in cases of disorders *of the external sphincter* but the evaluation in this respect was aggravated by the fact that the external sphincter had not been studied in cases with disorders of the internal sphincter Even if all the female cases with disorders of the internal sphincter at the same time were assumed to have disorders of the external sphincter, there would be no difference between the groups with and

without complications If the same assumption was applied to the men, however, disorders of the external sphincter would be found to occur more frequently in cases with complications

Table VII shows the relationships between complications and size and shape of the bladder It will be seen from the table that the men with complications more frequently had a large and atonic bladder than those without complications There was no such difference in the women None of the men with complications had a bladder of normal size and shape

Both men and women with complications more often had urinary retention The differences were almost significant Apart from this there was no apparent association

The relationship between the number of roentgenological changes observed (except sphincter disturbance and bladder bottom descent) and the presence or absence of complications has been scheduled in table VIII In male patients, there was on the average twice as many roentgenological changes when complications were present as when they were absent (2 9 and 1 5 respectively) The difference is significant The two men who showed changes of the internal sphincter but no clinical complications had two and three other roentgenological changes respectively apart from the internal sphincter disturbance As may be seen from the table the male patients with complications had one or more roentgenological changes

The tendency was the same among the women with a greater number of roentgenological changes when complications were present, but the difference is not significant (1 7 and 1 2 respectively)

In table II the relationship between duration of diabetes and the presence of complications is shown If the duration of diabetes in cases with none or only one apparent roentgenological finding is compared with the duration of the group showing two or more roentgenological changes it is found that in men the duration is significantly greater in the latter group This was not the case with the females

Discussion

The study population comprised cases from all stages of disease Many patients were hospitalized because of these and other investigations without any suspicions about so called late diabetic manifestations An over representation of these manifestations therefore have been avoided to the greatest possible extent

The frequency of peripheral neuropathy also points in this direction In this series it amounted to 49 % and as a comparison it can be mentioned that the corresponding figure in other series has been stated to be about 60 % (6 8 10 20) No investigation with the use of micturition urethrocystography of adult controls has been reported in the literature The study population has therefore been divided into cases with and without diabetic complications in the form of neuropathy or retinopathy

Symptoms from the urinary tract have been traced to a rate of frequency as high as 43 % but the majority of cases have been non characteristic In

about 9 % of the cases, the symptoms have, however, indicated disorders of neurogenic origin This figure is higher than that previously stated (14, 21) As pointed out before, urinary tract symptoms are discrete initially, but at a later stage they will be so pronounced that the patients ask for medical advice In order to gain a better conception of the frequency of non-characteristic urinary tract symptoms as well, we have used a special questionnaire and this detailed questioning of the patients is probably the reason for our frequency rates being higher than those stated in the literature The type of symptoms of the urinary tract indicate that the sensory parts of the peripheral nerves, the dorsal roots, and possibly also the dorsal tracts of the spinal cord may be injured The changes resemble those appearing in tabes dorsalis and should, for example result in impaired sensation of increased filling of the bladder Fagerberg et al (5), however, have demonstrated that peripheral diabetic neuropathy in other organs may also involve the motor function

In this population many roentgenological changes have been traced with great frequency primarily in the form of disturbance of the internal sphincter, large and atonic bladder as well as occurrence of urinary retention The findings agree to a considerable extent with those found with the aid of cystometry Several authors have also found that diabetic patients often, in addition to an increased bladder capacity display a diminished sense of the degree of bladder filling and a reduced detrusor function has also been observed (1, 7 12, 14, 18, 21, 23) In addition, Rudy and Muellner (18), on cystoscopy in one case, also found that the internal sphincter failed to open on voiding attempts

The roentgenological changes point to disorders of the sensory as well as the motor function of the bladder They have been traced more often in patients with neuropathy, but have also appeared in a number of cases without these complications The latter observation has previously been made in solitary cases only (11, 18, 21) The results thus appear to indicate, among other things, that neurogenic dysfunction of the bladder can comparatively often be traced even before it has been possible to establish any other neuropathy

Disorders of the internal sphincter as well as large bladder and abnormal bladder shape have been found significantly more often in men than in women This is rather remarkable, in view of the fact that the men had both lower average age and shorter duration of diabetes It has not been possible to trace the cause of these differences in the sexes, and the fact itself has never previously been mentioned in the literature

The presence of bacteriuria has been established to a great extent in our material, and has been related to cystitis, but not to roentgenological changes Theoretically speaking, however bacteriuria ought to be present more frequently in cases with neurogenic disorders particularly in cases with large atonic bladder and urine retention There is no therapy for the neurogenic bladder disturbance but in order to avoid complications due to infection

this should be subject to medical attention The sensory disorders probably result in the symptoms becoming apparent comparatively late Careful examination of the patient s history therefore constitutes a prerequisite for early application of therapy against infection In other respects, medical treatment is directed to the diabetes, and among other things this implies that the metabolic disorder is checked as carefully as possible Vitamins in large doses have been recommended mainly those included in the B-complex, but the effect is doubtful In certain cases the urinary retention may result in the need for catheterisation of the bladder In cases of patients having great difficulty in emptying the bladder surgical therapy may be indicated In three cases of this kind Emmet et al (4) has noted some improvement subsequent to re ection of the bladder neck Such measures have not been indicated in our cases

Summary

A material comprising 75 patients suffering from diabetes mellitus was clinically and roentgenologically examined regarding the presence of neurogenic disorders of the lower urinary tract Urinary tract symptoms were observed frequently — mostly in the form of cystitis Disturbances of the function of the internal sphincter large and atonic bladder as well as urine retention were frequently shown at roentgenological examination These changes were particularly numerous if the patients suffered from some other complication in the form of neuropathy or retinopathy In a number of cases, however, roentgenological changes were found even without any other definite complication The clinical symptoms as well as the roentgenological findings indicate a neurogenic dysfunction of the bladder of sensory as well as motor type The literature is referred to and the changes observed are discussed

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From Medical Department B (Head A Tybjærg Hansen) and the Department of Radiology (Head Gregers Thomsen) University Hospital (Rigshospitalet) Copenhagen Denmark

Chest Deformity in Patients with Congenital Isolated Ventricular Septal Defect

By

ERIK SANDOE, ALF WENNEVOLD and MOGENS EGEBLAD

Some patients with congenital heart disease present with a thorax of a peculiar shape resembling that which may be seen in children with chronic respiratory disease and emphysema The deformity consists of upper bilateral increase of the anterior posterior chest diameter with upper sternal protrusion and indrawing of the lower ribs, and it is easily recognized on a lateral chest roentgenogram (fig 1) The incidence of this chest deformity in children with congenital heart disease was first studied by Davies (2), and in the following we will use the term Ch D (Chest of Davies) when we refer to the deformity

The Ch D may occur in patients with different congenital cardiac diseases However, according to Davies (2) it is by far the most common in patients with ventricular septal defect (VSD) and it is especially seen in VSD with both pulmonary hypertension and arteriovenous shunt, i e in VSD with hyperkinetic pulmonary hypertension Davies (2 3) supposed that the lungs in such patients were abnormally stiff, and that this change in the mechanical properties of the lungs increased the respiratory forces on the thorax thereby leading to the deformation Lately two new series have been published which to some degree support the observations of Davies (1, 4)

The purpose of this paper is to re evaluate the incidence and the haemodynamic implications of the Ch D among patients with isolated VSD

Material and methods

The lateral chest roentgenograms of 84 patients with isolated VSD were reviewed by a radiologist (M E) and classified according to Davies (2) as showing severe deformity (fig 1) moderate deformity fig 2) or no deformity The reviewer had no knowledge of the haemodynamics of the patients.

The patients were part of a material of 87 patients previously published by one of us (5) The detailed haemodynamics are found in the mentioned monograph (5 to which the case numbers refer

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Fig 1 Lateral chest roentgenogram of 12 year old girl with severe chest deformity (case no 4)



Fig 2 Lateral chest roentgenogram of 8-year old boy with moderate chest deformity (case no 6?)

Three of the 87 patients were omitted from the present study because of lack of satisfactory lateral chest roentgenograms

Results

Three of the patients had severe Ch D (cases nos 4 59 and 80) while eight patients had moderate Ch D (cases nos 1 40 47 49 62 67 69 78 giving a total of 11 patients with the deformity

TABLE I Incidence of chest deformity in 84 patients with congenital isolated ventricular septal defect

Degree of deformity	No
Severe	3
Moderate	8
None	3
Total	84

(13 %) (table I) Ch D was present in nine of the 73 patients with left to right shunt (12 %)

As shown in tables II and III no relationship was found between the presence of Ch D and pulmonary hypertension or degree of shunt

Discussion

Davies (2) found Ch D in 21 of 31 patients with VSD and pronounced hyperkinetic pulmonary hypertension while it was only seen in one single case among 14 patients with VSD and slight or moderate pulmonary hypertension Corone and associates (1) found the deformity in 25 out of 27 patients with VSD and pronounced hyperkinetic pulmonary hypertension and in only 9 out of 37 patients with VSD and no or slight pulmonary hypertension Fi

TABLE II Relationship of chest deformity to the pressure in the pulmonary artery and to the shunt in 84 pat ents with ventricular septal defect

		Mean pressure n pulmonary artery			
		<25 (mm Hg)	26—49 (mm Hg)	>50 (mm Hg)	Total no
Flow rat o (FR)					
	>2 0	7 (2)	5 (0)	9 (1)	21 (3)
	1 1–1 9	37 (2)	2 (1)	13 (3)	52 6)
	0 4–1 0	—	—	11 (2)	11 2)
	Total number	44 (4)	7 (1)	33 (6	84 (11

Flow rat o = pulmonary blood flow/system c blood flow
The numbers in the table nd cate the numbers of patients in each group the numbers w thin brackets being those patients in each group with chest deform ty

TABLE III Relationsh p of chest deform ty to the pressure in the pulmonary artery and to he shunt in 73 pat ents w th ventr cular septal defect and predominant left to-right shunt

Haemodynam c cond t ons		No of pat ents <10 years old		No of pat ents >10 years old		Total no of pat ents	
Pressure (mm Hg)	FR	Total	W h ch est deform ty	Total	W th ch est deform ty	Total	W th chest d form ty
<25	1 1–1 9	19	1	18	1	37	2 (5°
<25	>2 0	4	0	3	2	7	2 29%
>25	1 1–1 9	8	3	7	1	15	4 (27%)
>25	>2 0	7	1	7	0	14	1 7%

Pressure mean pressure n pulmonary artery Table III cons sts of the two upper lines n table II rearranged as in table II in Polis & Sluyts (4)

nally Polis and Sluyts (4) encountered the Ch D in 12 out of 25 patients with VSD and hyperkinetic pulmonary hyperten sion and in only 1 of 28 patients with VSD and no pulmonary hypertension These series thus give the impression that the Ch D is very common among patients with VSD and hyperkinetic pulmonary hypertension In contrast to these observations we found a low incidence of Ch D in patients with VSD and furthermore the Ch D occurred without any relation to the haemodynam ics of the patients

We can offer no satisfactory explanation as to the difference between our series and those published by the other authors However, as the Ch D might exhibit a tendency to decrease (4) and the haemodynamics of the VSD to change during childhood and adult life (5, 6), one may pay some consideration to the age distribution in the different series In the series of Polis and Sluyts 63 % of the patients with pronounced pulmonary hypertension were below the age of 10 years, and one gets the impression that most of the patients in the series of Davies and Corone and associates were infants and young children 48 % of the patients in our series with pronounced hyperkinetic pulmonary hypertension were above the age of 10 years (table III) and none below the age of three years the Ch D was more common among the patients below the age of 10 years, but still not nearly as common as in the other series

The conclusion of our series will be that Ch D does occur in patients with VSD, but infrequently and without any definite haemodynamic implications at least after the age of three years

Summary

In a study of the lateral chest roentgenogram of 84 patients with ventricular septal defect a special chest deformity (Chest of Davies) was found in 11 patients (13 %) Of the 29 patients with pronounced hyperkinetic pulmonary hypertension the deformity was found in 5 (17 %)

We have been unable to confirm the relationship found by others between the presence of the deformity and hyperkinetic pulmonary hypertension

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From the Department of Medicine (Head S Samuelsson M D) University Hospital (Landspitalinn), Reykjavik Iceland

Chronic Rheumatic Valvular Heart Disease in Iceland

By

OLAFUR OLAFSSON[1] and JÓN STEFANSSON

The frequency of chronic rheumatic valvular disease of the heart among hospitalized patients in Iceland is reviewed in this report

It is difficult to assess the true frequency of chronic rheumatic valvular heart disease (R H D) in different countries since there have been few studies of representative populations Hall (18) reported the incidence of R H D in the population of the Swedish city of Malmo in five year periods from 1930 to 1954 The figures ranged from 2 7 to 2 0 per 10 000 and there was a 2 1 female preponderance Malmo has only one general hospital and non residents of the city were not accepted in Hall s material Autopsy is performed on 80 to 90 per cent of the patients who die in Swedish hospitals — figures probably among the highest in the world (5)

In Denmark Lindbjerg and Aastrup (22) found 412 cases of R H D during 20 year period in a population of 80 000 to 100 000 Clawson (11) reported 2 9 per cent in 30 265 autopsies in Minnesota (female male ratio 2 1) Cleland (13) reported the same figure from Australia Hall et al (19) stated an incidence of 4 5 per cent in a series from southern Sweden Gelfeman (17) reported 5 5 per cent from Boston but Bruno and Engelhart (9) found only 0 6 per cent in New Orleans Wood (31) estimated that 80,000 persons in Great Britain suffered from mitral stenosis Biorck (6) calculated from a series of clinical observations that 0 5 to 1 5 per cent of the Swedish population had R H D Some of his figures were based on personal observations and others on a mass radiographic survey in Stockholm (29)

Some writers (20 30) have found a diminishing frequency of R H D during the past 20 to 30 years Hall (18) showed this diminution to be statistically significant Lindbjerg and Aastrup (22) reported a decreasing incidence of mitral stenosis but stated that pure aortic valvular stenosis had become somewhat more common during the period 1940 to 1960

From Iceland Dungal (16) reported 18 cases of mitral stenosis in 2 200 autopsies performed between 1932 and

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[1] Present address Med thoraxklin Karolinska sjukhuset Stockholm 60 Sweden

1952, but in only one case was the lesion of rheumatic type. This report was reviewed by Coats and Biorck (14). Samuelsson and other clinicians (27), however, believe that R.H.D. may not be so rare in Iceland.

Own investigations

We have reviewed the cases of R.H.D. treated at the medical department of the University Hospital in Reykjavik, Iceland during the period 1949 through 1963. The medical department has 58 beds. The University Hospital is one of the three hospitals in Reykjavik (population ca. 54,000 in 1949 and ca. 75.000 in 1963). About one third of the cases accepted at the University Hospital are referred from other parts of the country (total Icelandic population during the study period ca. 141.000—180.000). In consequence the population from which our clinical material was drawn cannot be reliably estimated.

The autopsy rate at the University Hospital is more than 90 per cent.

All the cases with a diagnosis of R.H.D. were studied. We excluded cases in which the diagnosis was uncertain, and also those with syphilis, congenital heart defects or endocarditis without previous clear evidence of valvular disease.

There remained 90 patients who had a diagnosis of mitral stenosis with or without incompetence, aortic valve stenosis with or without incompetence or combined mitral and aortic lesions.

The cases were classified according to the following criteria (1, 2), which are similar to those recommended by the New York Heart Association (25).

Mitral stenosis

Diastolic or pre-systolic murmur over the cardiac apex (in left lateral position).

Mitral incompetence

Pan-systolic murmur, best heard in the apical region and left axilla, and signs of left ventricular hypertrophy.

Aortic stenosis

Loud systolic murmur in the second right intercostal space or sternal region, radiating over the carotid arteries or to the right axilla. In addition, at least two of the following three signs were required for this diagnosis: 1) thrill over the aorta, 2) second aortic sound weak or inaudible in the first and second right intercostal space, 3) pulsus tardus.

Aortic incompetence

Diastolic murmur in the second right intercostal space, over the sternum or in the third to fourth left intercostal space.

Combined aortic and mitral stenosis

In doubtful cases the diagnosis was based upon the dominant murmur.

All the patients were clinically examined be specialist cardiologists. We personally examined the cases from the later years of the study. The examination comprised ECG (at first 4 leads and later during the observation period 12 leads), chest X-ray, measurement of blood pressure, Wassermann tests, ESR and full blood counts. Phonocardiography was carried out in some cases.

Electroradiographic and roentgenographic findings (left atrial or ventricular enlargement) were accepted as supportive diagnostic evidence. In order to obtain uniformity of the criteria, however, the diagnosis was based mainly on auscultation, since 12 lead ECG and lateral roentgenologic views were not used in the early years of the study.

Results and discussion

The distribution of the clinical material according to diagnosis, age and sex is shown in table I, which also records the number of patients submitted to operation and/or heart catheterization and the number of autopsies. It is seen that the male : female ratio was 33 : 57. In mitral disease this ratio was 9 : 41,

TABLE I Distribution of the cases according to age and sex

Diagnosis		Age in years							Operation and/or catheterization	Autopsy
		20–29	30–39	40–49	50–59	60–69	> 70	Total		
Mitral stenosis or incom	♂	3		1	3	2		9	5	2
petence	♀	1	6	12	14	7	1	41	22	7
Aortic stenosis or incom	♂	1	3	5	3	2	3	17	3	1
petence	♀			1	1	5	1	8		
Combined mitral and	♂			1	4	2		7	1	3
aortic disease	♀	1		1	1	4	1	8	1	5
Total								90	32	18

in aortic valvulopathy 17 8 and in combined valvular lesions it was 7 8

The male female percentage ratio (no of males and no of females per 100 persons) in the total series was 37 63 Hall (18) reported 33 67 in his clinically examined series and Lindbjerg and Aastrup (22) found 39 61 in cases examined clinically and/or post mortem In mitral disease the male female percentage ratio in our cases was 20 80 Hall gave identical figures whereas Lindbjerg and Aastrup found 30 70 The corresponding ratio in aortic disease in the present series was 32 68 Hall stated 30 70 and Lindbjerg and Aastrup 46 54 In combined mitral and aortic disease the male female percentage ratio in our series was 53 47, as compared with Hall s 71 29 and 32 68 reported by Lindbjerg and Aastrup

Thirty two of our patients underwent operation and/or heart catheterization and 18 others were examined post mortem It was not possible to obtain satisfactory information from all the autopsies performed on cardiac cases during the period of the study The accuracy of clinical diagnosis as represented by the 50 cases in which the diagnosis was confirmed by catheterization, operation or autopsy was 87 per cent Lindbjerg and Aastrup (22) reported 92 4 per cent in this respect

The diagnosis of mitral incompetence is difficult (8) Of the 50 patients with mitral disease only 3 were classified as having isolated valvular incompetence Biorck et al (7) reported a similar frequency, viz , 6 of 134 patients In one of our cases the diagnosis of mitral incompetence was confirmed by heart catheterization, but in the other two it was based on a loud coarse pan systolic murmur in the region of the cardiac apex and the axilla with distinct widening of the apex and roentgenologic enlargement of the left ventricle Many of the patients with

TABLE II Rheumatic fever in the case history of chronic rheumatic valvular heart disease

	Present series				Landbjerg & Aastrup (27)
Diagnosis	Sex	No of patients	No with rheumatic fever	% with rheumatic fever	% with rheumatic fever
Mitral stenosis or incompetence	♂	9	6	54	47 2
	♀	41	21		
Aortic stenosis or incompetence	♂	17	7	44	25
	♀	8	4		
Combined mitral and aortic disease	♂	7	4	73 3	69 5
	♀	8	7		
Total		90	49	53 3	43

mitral stenosis also had a relatively weak or brief systolic murmur over the cardiac apex

The pulse pressure was rather high in some cases of aortic valvular stenosis but it is known that up to 20 per cent of these patients are hypertensive (2) In a few cases of this group only the character of the murmur was noted at the initial examination, but the diagnosis was later confirmed by heart catheterization or autopsy Only a very few patients had "pure" aortic valvular incompetence Some of those with aortic stenosis showed mild signs of valvular incompetence This group possible contained occasional cases of subvalvular aortic stenosis

There were 15 cases of combined mitral and aortic valvular disease We have not attempted to subdivide this group

In earlier years many writers tried to differentiate pathologically and clinically between aortic valvular disease of rheumatic origin and degenerative stenosis or calcification of the aortic valve (24) More recent investigations have shown that rheumatic changes of varying degree are present in almost all stenosed or calcified aortic valves (12 21, 23, 33) That patients with aortic valvular stenosis less commonly give a history of rheumatic fever than do patients with mitral stenosis is possibly attributable to the considerably higher age of the former group when they seek medical advice, and hence to their less reliable memory of earlier illnesses

Rheumatic fever

We searched all the case records for information on rheumatic fever In addition to documented rheumatic fever we accepted as positive diagnostic criteria tonsillitis in combination with acute polyarthritis or cardiac symptoms Cases with epistaxis and tonsillitis alone were not accepted As our series was collected from 1949 onwards the diagnostic criteria published by the American

TABLE III Anatomic distribution of chronic rheumatic valvular heart disease

	Period of study	No of cases	Mitral stenosis or incompetence (%)	Aortic stenosis or incompetence (%	Combined mitral and aortic disease
Present study	1949–63	90	55 0	27 8	16
Hall (18)	1953–58	229	58 5	17 5	24 0
Lindbjerg & Aastrup (22)	1940–60	412	38 6	38 5	23 0
Cullhed (15)	1964	397	57 0	25 0	18 0
Aastrup et al (2)	1963	—	c 50	15–20	20 [illegible]
Cecil & Loeb (10)	1959	—	c 50	20–30	20

Heart Association in 1955 (3) could be used only in the later part of the study

Table II shows the calculated incidence of rheumatic fever in our cases of R H D and in the series reported by Lindbjerg and Aastrup The respective percentages in the total series were 53 3 and 43 In the various constituent groups the figures were similar except in aortic valvular disease where the percentage of patients who had rheumatic fever was higher in our study than among the cases described by Lindbjerg and Aastrup Warburg (28) Cecil and Loeb (10) and Olesen (26) found that 50 to 60 per cent of patients with chronic rheumatic cardiopathy had a history of rheumatic fever Mitchell et al (23) and Anderson (4) however, reported that 34 to 58 per cent of patients with aortic stenosis or incompetence had had rheumatic fever and Cecil and Loeb (10) gave a corresponding frequency of 30 per cent

Anatomic distribution of the lesions

Of the 90 patients in our series, 55 5 per cent had mitral disease 27 8 per cent had disease of the aortic valve and 16 7 per cent had combined mitral and aortic lesions In table III these figures are compared with frequencies reported by some other writers

Our findings were in agreement with reports by Cullhed (15 Aastrup et al (2) and Cecil and Loeb 10 Hall 18 however found a higher percentage of combined lesions and a lower percentage of aortic lesions in his clinically examined series In the cases described by Lindbjerg and Aastrup 22 and examined clinically and or post mortem the frequency of mitral disease was lower than in our series and the frequency of aortic disease was higher

Summary and conclusions

The frequency of chronic rheumatic valvular heart disease (R H D) was studied in patients hospitalized in the medical department of the University Hospital in Reykjavik Iceland between 1949 and 1963 This diagnosis was accepted as having been confirmed in 90 cases

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From Medical Department C (Head Aage Videbæk M D) Gentofte Hospital, Copenhagen Hellerup Denmark

Use of a Methyl Hydrazine Derivative (Natulan[R]), Especially in Hodgkin's Disease

By

M Mork Hansen, H Hertz and Aa Videbæk

Natulan[R] (1 methyl 2 p-(isopropylcarbamoyl) benzylhydrazine) is a new cytostatic having a mechanism of action which differs fundamentally from that of previously known chemotherapeutics Since the autumn of 1962 this substance has been tested on humans, and the results though still rather few, appear to be promising in malignant diseases of the lymphoreticular system especially in Hodgkin s disease (1—11)

Our experience and results of treatment with Natulan will be reported below

Material and administration

This series comprises 35 patients who were treated for a varying length of time with Natulan since December 1963 Out of this group 14 had Hodgkin s disease 6 reticulosarcomatosis 1 lymphatic leukaemia 1 lymphosarcomatosis 1 follicular lymphoma and 12 solid tumours. All patients who recevied more than 1 000 mg of the drug have been included in the series

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In all the cases but 2 (cases 7 and 18) Natulan was initially administered intravenously and the total parenteral dose if all given was 5—6 g The dry material was delivered in a 250 mg ampoule and was dissolved immediately before use in 10—20 ml re-distilled water or in physiological saline On the first day 250 mg was administered on the next day 500 mg and thereafter 1 000 mg every other day until — in the course of 10—12 days — the desired total dose had been attained In a few cases severe nausea or signs of bone marrow depression made it necessary to reduce the single or total dose For administration of 1 000 mg the solution of the substance was introduced close to the vein by way of the infusion tube of an established saline or glucose drip and thereafter rinsed with 100—200 ml of the infusion solution at a rapid drop rate In 16 cases the treatment was repeated after a pause of 3—4 weeks and in these cases a maintenance therapy of 50 mg capsules in doses of 50—150 mg daily was given while the white cell and platelet counts were checked On signs of recurrence of the disease the oral dose was in some cases increased up to 300 mg daily

TABLE I Results of treatment in Hodgkin s disease In assessing the value of Natulan it must be mentioned in respect of the duration of the remissions that after the Natulan therapy the patients always enjoyed complete well being (often for several months) beyond the period in which all the criteria of complete remission were fulfilled

			Dose of Natulan(g)				
Case no	Sex	Age	Before remission	Total	Time of observation from 1st day of treatment (months)	Result[1]	Duration of full remission (months)
1	♀	23	$2^{3}/_{4}$	26	19	++	10
2	♀	39	$5^{3}/_{4}$	$17^{1}/_{2}$	12 (†)	+	
3	♀	35	10	$49^{1}/_{4}$	17	++	1
4	♀	38	$5^{3}/_{4}$	$18^{1}/_{2}$	8	++	>8
5	♂	21	$5^{3}/_{4}$	29	$9^{1}/_{2}$	++	$1^{1}/_{2}$
6	♂	65	$3^{3}/_{4}$	9	$1^{1}/_{2}$	+	
7	♂	10	4	$6^{1}/_{2}$	2	++	>$1^{1}/_{2}$
8	♂	25	$5^{3}/_{4}$	$19^{1}/_{4}$	$4^{1}/_{2}$	+	
9	♀	26	$5^{3}/_{4}$	$9^{3}/_{4}$	11	++	2
10	♀	24	$4^{3}/_{4}$	$24^{3}/_{4}$	9	++	2
11	♀	37	—	$6^{3}/_{4}$	11	~	
12	♂	47	$5^{3}/_{4}$	$32^{1}/_{4}$	8	++	2
13	♀	36	$6^{1}/_{4}$	8 /$_{2}$	1	+	
14	♂	32	$5^{3}/$	$32^{1}/_{4}$	12	++	>12
Average		33	$5^{1}/_{2}$	$20^{2}/_{3}$	9		

[1] ++ full remission
+ partial remission
~ unassessable

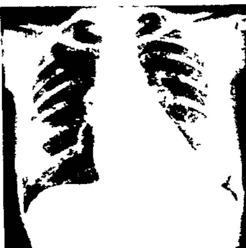
Results

Hodgkin s disease

Altogether 14 patients 8 females and 6 males, in the age range 10—65 years were treated The diagnosis was confirmed histologically in all cases The indication for treatment with Natulan was in all cases a poor general condition and signs of generalization Only 4 of the patients had not previously been treated with X rays, prednisone and/or other cytostatics All the patients were continued on oral doses of the drug after the parenteral treatment, and a few also received another 2 or 3 series of parenteral treatment The total dose was between 6 3/4 and 49 1/4 g, average 20 g

As criteria of a complete remission we demanded

1 Diminution of lymphomas and of the liver and/or spleen, if enlarged



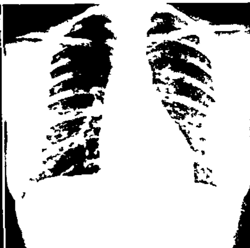


Fig 1 Fig 2

Fig 1 Case 1 X ray appearances of the chest prior to treatment with Natulan.

Fig 2 Case 1 X ray appearances of the chest 3 months after institution of Natulan therapy The hilar changes have almost completely disappeared

2 Complete disappearance of symptoms and weight gain in the event of underweight

3 Erythrocyte sedimentation rate $\leqslant$ 30 mm

4 Haemoglobin $\geqslant$ 12 g/100 ml

5 Normal temperature

In determining the duration of complete remission, we included only the period during which all 5 criteria were fulfilled If the criteria of complete remission had been fulfilled for only 2—4 weeks the case was interpreted as partial remission Furthermore, the designation partial remission was used for patients who after Natulan therapy fulfilled item 1 and showed marked improvement of symptoms Less than 2 weeks effect of the treatment was not listed as a remission

Nine of the 14 patients went into complete remission and 4 showed partial remission In one case (no 11) the result was unassessable, as during the treatment with Natulan the patient was started on prednisone

The duration of complete remission ranged from 4 weeks to 12 months 3 patients with 1 1/2 8 and 12 months remission (cases 4 7 and 14) were still in remission at the time of writing (cf table I)

Case reports

Case 1 A woman born in 1940 The diagnosis had been made on the basis of a biopsy in 1960 From 1960 to 1963 she had been admitted several times and treated with X rays chlormethine (Erasol[R] chlornaphazine (R 48) vinblastine (Velbe[R]) prednisone and numerous blood transfusions The general condition as well as the laboratory findings were rather fluctuating showing a short lasting effect each time a new cytostatic was tried but on the whole there was progression with fever a tendency to sweating and weight loss

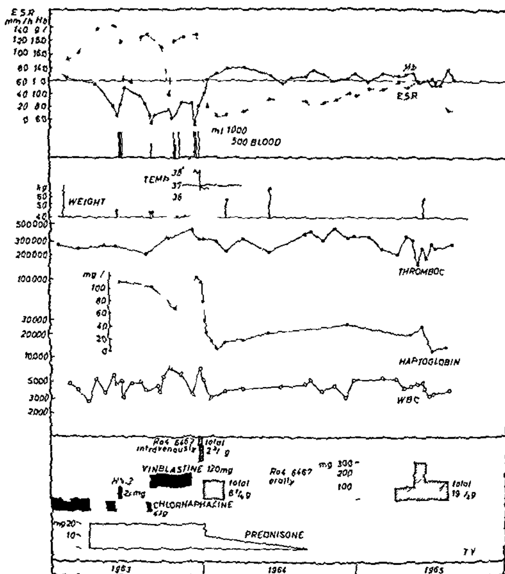


Fig 3 Laboratory findings and treatment in a case of Hodgkin s disease (case 1) Natulan (R04-6467) was given i v for a short initial period and by mouth for two periods each of several weeks duration

In December 1963 she was admitted in a state of fever cachexia and anaemia, with a high E. S R and impaired hepatic function There were only a few peripheral lymphomas but the superior mediastinum was increased in width and the right hilus was enlarged Moreover she had moderate spleno and hepatomegaly From March 1963 she had been on continuous prednisone medication The patient was started on Natulan and during this treatment she went into complete remission and the E S R Hb temperature and hepatic function returned to normal The changes in the chest disappeared almost completely (cf figs 1 and 2) and the hepato-splenomegaly dis

appeared Prednisone could then be discontinued and the patient put on 23 kg in 6 months She was feeling perfectly well

At the end of 10 months there were signs of recurrence in the form of an increasing E S R a slightly falling Hb and in March 1965 she was again tired and feverish However after another course of Natulan therapy she again went into complete remission which is still being maintained at the time of writing (Sept 1965) (cf fig 3)

Case 2 A woman born in 1925 The diagnosis was based on a biopsy in 1961 From 1961—1963 she was admitted several times and treated with X rays and chlornaphazine

During a stay in hospital in February 1964 Natulan was started because of recurrent fever itching and a tendency to sweating lymphomas on the neck and in the axillae E S R 128 mm Hb 9 8 g/100 ml For a couple of months after this treatment she was feeling well had a normal temperature and the peripheral lymphomas had disappeared The E S R fell to 70 mm and the Hb increased to about 11 g/100 ml Owing to thrombocytopenia with cutaneous haemorrhages prednisone was administered in April 1964

From the summer of 1964 she was again declining and she died in hyperpyrexia in February 1965 despite several new attempts at treatment int al with Natulan

Case 3 A woman born in 1929 The diagnosis was based upon a biopsy specimen in 1942 and at that time the patient was treated with X rays Thereafter she kept symptom free until 1959

From 1959—1963 she was admitted several times and treated with X rays chlormethine and vinblastine

In January 1964 she was admitted in hyperpyrexia with a few lymphomas on the neck and epigastric swelling E S R 133 mm Hb 10 g/100 ml After treatment with Natulan the temperature returned to normal the lymphomas disappeared and the E S R as well as Hb values returned to normal The patient was feeling well

From August 1964 she was again prone to fatigue and had an elevated E S R During a repeated Natulan therapy there was again subjective improvement but only a moderate decrease in the E S R Despite a continued Natulan therapy for 6 months supplemented with prednisone because of marked fatigue there was on the whole a steady progression of the disease and when gradually thrombocytopenia appeared Natulan was discontinued in February 1965

Case 4 A woman born in 1926 The diagnosis had been based upon a biopsy specimen in May 1964 She was treated with X rays

In December 1964 treatment with Natulan was instituted because of weight loss a tendency to sweating low grade fever and slight anaemia Chest radiography showed a widened superior mediastinum and an enlarged left hilus The liver as well as the spleen were 4—5 cm below the costal border During the treatment the temperature returned to normal the sweating tendency yielded and the patient felt perfectly well Three weeks after the Natulan therapy a short course of prednisone was administered because of pronounced thrombocytopenic purpura Since that time the patient continued on Natulan until June 1965 when the treatment was interrupted because of renewed thrombocytopenia and cutaneous haemorrhages She is still feeling perfectly well and shows no signs of recurrence (cf fig 4)

Case 5 A male born in 1943 The diagnosis was made by biopsy in 1961 From 1961—1964 he had been admitted a few times and treated with X rays cyclophosphamide and prednisone

In August 1964 he was treated with Natulan because of elevated temperature a tendency to sweating weight loss bone pain and anaemia E S R 120 mm Only scattered small lymphomas peripherally During the treatment the subjective symptoms yielded he gained weight and the E S R as well as Hb returned to normal

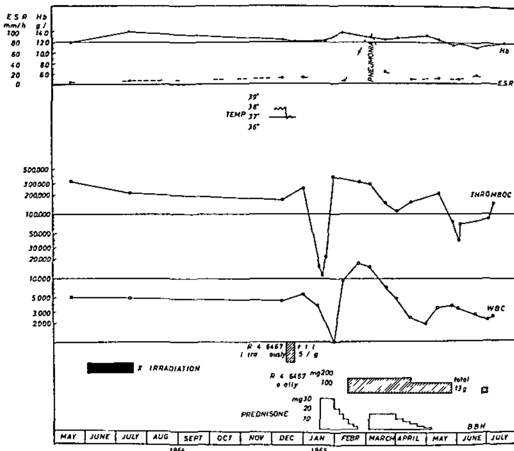


Fig 4 Laboratory findings and treatment in a case of Hodgkin s disease (case 4) Note the pronounced leukocytopenia and thrombocytopenia which appeared 3 weeks after discontinuation of intravenous Natulan (R04 6467) therapy

At the end of 2 months he had a recurrence, and since that time the patient has periodically been treated with Natulan with some effect In the summer of 1965 he was feeling perfectly well with a normal E S R and Hb but owing to failing co operation on his part, he had just been shifted to another cytostatic (cyclophosphamide) It is therefore difficult to assess the possible share of Natulan in this remission

Case 6 A male born in 1900 The diagnosis had been made on the basis of a biopsy specimen in February 1965

Natulan therapy was instituted in June 1965 because of episodes of fever and fatigue A few lymphomas in the axillae impaired hepatic function a high E S R and slight anaemia During the treatment the temperature E S R and hepatic function returned to normal while some anaemia persisted Already at the end of 3 weeks during continued Natulan therapy the E S R was rising but still he is gaining weight his temperature is normal and he is feeling relatively well

Case 7 A boy born in 1954 The diagnosis was made on the basis of a biopsy in March 1964 From March 1964 to May 1965 he was treated with X rays *cyclophosphamide* chlormethine prednisone, and blood transfusions

From the middle of May 1965 he was started on Natulan because of a poor general condition a fluctuating temperature anaemia a moderate increase in E S R, hepato-splenomegaly and a widened mediastinum During the treatment he gradually attained complete well being The Hb and E S R returned to normal and the fluctuations in the temperature stopped The mediastinal lymphomas as well as the liver and spleen decreased in size Prednisone which had been administered in a high dosage through 6 months int al because of pronounced haemolysis was levelled off Three months later he was feeling well

Case 8 A male born in 1939 The diagnosis had been made on the basis of biopsy in 1962 From 1962 until February 1965 he was treated with X rays and cyclophosphamide

Owing to a poor general condition with cachexia a sweating tendency fever and bone pain (destructions in the lumbar column and pelvis) Natulan was instituted in March 1965 During this treatment there was a considerable improvement in the general condition and the temperature returned to normal During the subsequent 2 months he gained 9 kg The E S R dropped to half its former level the Hb slowly rose and abdominal lymphomas disappeared At the end of 5 months he was still feeling fairly well and was working full time

Case 9 A woman born in 1938 The diagnosis had been made on the basis of a biopsy in August 1964

Owing to an increasing fatigue tendency to sweating itching and a dry cough the patient was started on Natulan During this treatment she felt well her E S R fell from 83 mm (21 8) to 16 mm (9 9) Prior to the treatment lymphomas had been demonstrated in the mediastinum and right hilus but had now disappeared

Towards the end of October 1964 there were increased radiological changes in the chest and a rising E S R so that in December 1964 Natulan was administered again Again there was a regression of the radiological changes and the E S R returned to normal

Case 10 A woman born in 1940 The diagnosis had been made on the basis of biopsy in 1960 From 1960 to December 1963 she was treated with X rays and chlornaphazine

In October 1964 she was admitted with a dry cough and pain in the right chest X ray examination revealed infiltration in the right lung and in addition conglomerates of lymphomas were found on the neck and in the axillae E S R 96 mm She was started on Natulan and thereafter felt well The radiological changes as well as the peripheral lymphomas have considerably decreased E S R returned to normal

Case 11 A woman born in 1927

From February 1964 she was suffering from increasing fatigue and a great weight loss and gradually episodes of fever On admission in August 1964 she had lymphomas on the neck in the left hilus and in the superior mediastinum E S R 130 mm and the temperature in the evenings was 38 5° C At that time the diagnosis was made on the basis of biopsy She was started on Natulan which was supplemented at the end of 12 days with prednisone because of a poor general condition with persisting elevation of temperature

In October 1964 she was feeling perfectly well gaining weight and the lymphomas were regressing The temperature had returned to normal but the E S R and Hb values had not appreciably improved

No attempt was made to assess the value of Natulan in her partial remission because of the simultaneous treatment with prednisone

Case 12 A man born in 1916 The diagnosis had been made on the basis of a biopsy in November 1964

Prior to admission in November 1964 he had been suffering from herpes zoster of the face and had noticed an intolerance to alcohol During the past 6 months he had been increasingly bothered by sweating

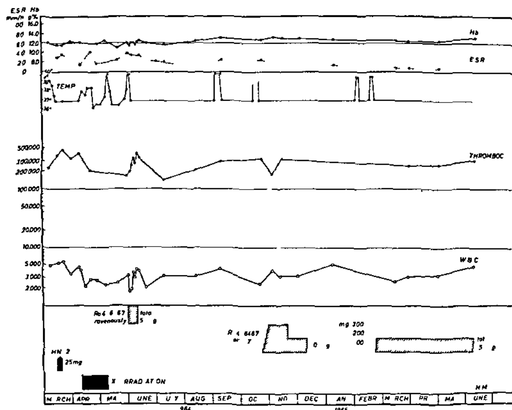


Fig 5 Laboratory findings and treatment in a case of Hodgkin s d sease (case 14) Note the d s-appearance of the fever (Pel Epstein type) each time the drug was administered

itching and fatigue On admission he had an elevated temperature lymphomas on the neck in the axillae and groins and an E S R of 81 mm Chest radiography blurring of the costophrenic sinus After Natulan therapy there was improvement of all symptoms and a normal temperature One month later there were no palpable peripheral lymphomas the chest radiography was normal and the patient was feeling perfectly well His E S R was normal

Owing to a recurrence of the peripheral lymphomas and an elevated E S R another course of Natulan was started in May 1965 and again the lymphomas decreased In the middle of July 1965 he was still feeling well

Case 13 A woman born in 1928 The diagnosis had been made on the basis of biopsy in November 1963 From November 1963 to October 1964 the patient had periodically received X ray irradiation of peripheral lymphomas From October 1964 she complained of interscapular pain radiating down the back but physical examination and X rays did not reveal any changes of the spine Owing to the pain X ray therapy to the spine (3 000 r) was administered in April 1965 but without any effect

In May 1965 the patient was admitted because of difficulty in walking which had been rapidly progressing for the past 2 weeks Now X ray examination of the dorsal column revealed destruction of $Th_{3\ 4}$ and physical examination showed a pronounced spastic inferior paraparesis with hypaesthesia and analgesia from Th_3 and distal Within a few days there was total paralysis of the lower limbs and incontinence of faeces and urine

TABLE II Results of treatment in 9 patients with malignant lymphoma of types other than Hodgkin's disease

Case no	Diagnosis	Sex	Age	Dose of Natulan (g) Before remission	Total	Time of observation from 1st day of treatment (months)	Result[1]
15	Reticulosarcomatosis	♀	43	$5\frac{1}{4}$	$28\frac{1}{4}$	8	+
16	Reticulosarcomatosis	♂	72	$5\frac{3}{4}$	$7\frac{1}{4}$	$4\frac{1}{2}$	+
17	Reticulosarcomatosis	♀	74	—	$3\frac{1}{2}$	4	—
18	Reticulosarcomatosis	♂	9	—	$5\frac{1}{2}$	$1\frac{1}{2}$ (†)	—
19	Reticulosarcomatosis	♀	80	—	$4\frac{3}{4}$	$\frac{1}{2}$ (†)	—
20	Reticulosarcomatosis	♀	58	—	$4\frac{3}{4}$	1 (†)	—
21	Leukaemia lymph chron	♀	72	—	$4\frac{1}{2}$	$1\frac{1}{2}$ (†)	—
22	Lymphosarcomatosis	♂	77	$5\frac{3}{4}$	$5\frac{3}{4}$	3 (†)	+
23	Follicular lymphoma	♂	47	—	$3\frac{3}{4}$	$\frac{1}{2}$ (†)	—
Average			59	$5\frac{3}{}$	$7\frac{1}{2}$	$3\frac{1}{2}$	

[1] + partial remission
— no effect

Owing to a suspicion that granuloma formation was a contributory pathogenetic factor in the cord compression Natulan therapy was instituted and as early as 2 weeks after the first dose it began to have an effect The paresis decreased the sensibility returned and the incontinence ceased

Case 14 A male born in 1932 The diagnosis had been made on the basis of a biopsy in March 1964 From March to June 1964 he was admitted twice and treated with chlor methine and X rays respectively

From December 1963 he complained of fatigue and a tendency to sweating as well as recurrent episodes of high fever lasting for about 5 days at about two-week intervals (Pel Epstein) Because of a recurrence of fever and other symptoms (after a very short lasting effect of the above mentioned treat ment) the patient was again admitted and treated with Natulan in June 1964 There after, his febrile episodes ceased and he was feeling perfectly well When the Natulan medication was discontinued the febrile attacks recurred but again yielded com pletely when the treatment was resumed (cf fig 5)

Reticulosarcomatosis

Six patients with histologically confirmed reticulosarcomatosis were treated All were in a poor general condition, showing signs of generalization Their sex and age are shown in table II None of these patients went into complete remission (cf the above mentioned criteria) Two (cases 15 and 16) obtained a partial remission One of these two patients has been on continuous medication receiving

TABLE III Results of treatment in 12 patients with various metastasizing malignant tumours

Case no	Diagnosis	Sex	Age	Dose of Natulan (g) Total	Time of observation from 1st day of treatment (months)
24	C sol bronch metast	♀	70	$5^{3}/_{4}$	2 (†)
25	C sol bronch metast	♂	66	$4^{1}/_{4}$	$^{3}/_{4}$ (†)
26	C sol bronch metast	♂	67	$8^{3}/_{4}$	$3^{1}/_{2}$ (†)
27	C anaplast pulm	♂	63	$5^{3}/_{4}$	$1^{1}/_{2}$ (†)
28	Mesothel ovarii metast	♀	53	$4^{3}/_{4}$	$^{1}/_{2}$ (†)
29	Adenocarc ovarii metast				
	C sol mammae	♀	64	3	1 (†)
30	Adenocarc ovarii metast	♀	72	$5^{3}/_{4}$	$^{3}/_{4}$ (†)
31	Chorionepith test metast	♂	24	$11^{1}/_{2}$	3 (†)
32	Melanom malign metast	♂	56	22	4 (†)
33	Melanom malign metast	♀	68	$5^{1}/_{4}$	1 (†)
34	Thymoma malign	♀	76	$3^{1}/_{4}$	$^{1}/_{2}$ (†)
35	C sol mammae metast	♀	54	$4^{3}/_{4}$	$^{1}/_{2}$ (†)
Average			61	$7^{1}/_{4}$	$1^{1}/_{2}$

a low maintenance dose for 8 months, and is feeling better on this treatment than previously, never during this period has the patient required admission to hospital The oral maintenance treatment of the other patient, who gave a partial response to the treatment had to be discontinued at the end of a few weeks because of pronounced leukocytopenia (800/μl) and thereafter the condition again deteriorated One patient (case 17) received only 3 1/2 g Natulan, whereupon the treatment had to be interrupted because of exanthema and elevated temperature, reproduced upon an attempt at resuming the treatment Three of the 6 patients died a few weeks after the treatment because of progression of the disease (cf table II)

Lymphatic leukaemia, lymphosarcomatosis, giant follicular lymphoma

One patient with chronic lymphatic leukaemia (case 21), diagnosed 2 years previously, was treated because of anaemia and pronounced hypermetabolism Previously, an attempt had been made to improve the condition by prednisone and chlorambucil In this case Natulan was ineffective One patient (case 22) with lymphatic leukaemia and lymphosarcomatosis was treated because of rapidly growing abdominal lymphomas and an enlarged liver which caused abdominal pain For 3 weeks after the treatment the patient had no pain and there was less swelling in the abdomen, but thereafter the disease rapidly progressed (cf table II)

Solid tumours

Twelve patients with various malignant tumours, nearly all with metastases, were treated with intravenous injections of Natulan. All were in an extremely poor general condition and died, shortly after the treatment, from progression of their disease without any definite effect of the drug being demonstrable (cf. table III). In one patient (case 32), with a metastasizing melanosarcoma, however, an improvement in the general condition and a considerable decrease in E. S. R. were obtained, but at the end of 4 weeks the disease again quickly progressed despite renewed administration of Natulan.

Side effects

The side-effects of Natulan therapy consisted mainly of nausea and bone marrow depression, but other untoward effects were also observed.

Nausea

Nausea and vomiting occurred frequently, probably due to a toxic effect of Natulan upon the central nervous system.

As already mentioned, the majority of the patients in the group with solid tumours were extremely debilitated before being started on the treatment. Eight of these patients had gastrointestinal complaints in the form of nausea or vomiting before the treatment. Out of 25 patients 18 had nausea and 14 also vomiting during the first hours after intravenous administration of Natulan. However, it was in only a very few cases that the single doses had to be reduced for this reason and in no case was the treatment interrupted.

To combat the nausea the patients usually received pyridoxine during the period of the intravenous injections and furthermore a few patients were given a small dose of chlorpromazine (25 mg) a short time before each injection — often with a good effect. Five of the 18 patients who received oral treatment complained of nausea and 3 of them also had vomiting. Many received prophylactic pyridoxine simultaneously with oral Natulan, and at a daily dose of 150 mg or over the drug was administered in smaller single doses, usually 3 divided doses in the 24 hours.

None of the patients had diarrhoea.

Bone marrow depression

After intravenous administration of 5—6 g Natulan leukocytopenia (< 2 000/μl) and thrombocytopenia (< 100 000/μl) were common. These changes usually did not occur until 2 or 3 weeks after the cessation of intravenous therapy. Ten of the patients died within 3 weeks after the last intravenous dose, and out of these patients only 2 had time to develop leukocytopenia and thrombocytopenia. (Both patients (cases 20 and 23) had been treated immediately before the Natulan therapy with X rays and one of them also with chlorambucil.) Twenty three patients were alive more than 3 weeks after the last intravenous dose. Of this group 11 developed leukocytopenia and 11 thrombocytopenia. In one patient (case 4) the platelet count fell to 10 000/μl and she developed cutaneous haemorrhages. For a period she was therefore treated with prednisone. During a subsequent oral course this patient again developed severe thrombocytopenia with cutaneous haemorrhages (cf. fig. 4).

In all cases the leukocytopenia and thrombocytopenia disappeared within 4 weeks after discontinuation of Natulan and irreversible bone marrow damage was not seen.

The unwanted depression of the leukocyte and platelet formation appeared to be accentuated and to occur earlier if the patients had been treated prior to the Natulan therapy with other cytostatics or X rays. Moreover, there was perhaps an individual difference in the tolerance to Natulan.

One patient (case 18) in the terminal stage of reticulosarcomatosis received simul

taneously with oral Natulan, X ray irradiation to a large subcutaneous metastasis, this patient developed severe pancytopenia also with considerable depression of the erythropoiesis Otherwise none of the patients showed any material decrease in haemoglobin during or immediately after treatment with Natulan In several instances there was mild reticulocytosis in the blood during the treatment and in some cases there was a considerable fall in the haptoglobin concentration The fall in haptoglobin was particularly evident in patients with Hodgkin's disease who as is usual, had a high haptoglobin content in the blood prior to the treatment In some cases there was a fall to 0 or almost 0 mg/100 ml presumably indicating partly a decreasing disease activity and partly subclinical haemolysis The haemolysis was not in any case so severe that the patients could not compensate for it and there was no instance of an increase in serum bilirubin

Other side effects

An elevation of temperature up to 40° C and a generalized delicate rash occurred in three patients after the 5th, 5th and 6th intravenous dose respectively The temperature returned to normal in a few hours and the rash disappeared at the end of a day or two In one of the patients the reaction was extremely violent, and for a few hours he was sweating and delirious In this case therefore, hydrocortisone was administered in addition to antazoline A lowered blood pressure or a flush syndrome were not observed in any of these cases The reaction did not manifest itself until 4—5 hours after the injection and two of the patients were treated during the subsequent days orally with 50 mg Natulan daily without side effects In no case did we observe blood eosinophilia on the day after the reaction On attempts at repeating the injection therapy in 2 of the patients (250 mg Natulan) the reaction occurred again One of these patients has since that time been able to continue on Natulan up to 200 mg daily by mouth without any complaints other than nausea

Two patients complained of dizziness and drowsiness and after the 5th intravenous injection of Natulan (1 000 mg) one patient showed a lowering of blood pressure to 70 mm systolic with clinical signs of shock a few minutes after the injection However this patient rallied spontaneously in the course of 15—30 minutes The treatment was then continued orally, in a dose of up to 300 mg daily, which was tolerated without untoward effects This patient did not receive any other drugs before or during the injection therapy

No mental reactions such as confusion euphoria, or depression were observed

Lastly it may be mentioned that no patient complained of hair loss and that the severity of diabetes mellitus in 2 patients on insulin did not alter during the Natulan therapy

Discussion

Our results of Natulan therapy are in good accordance with previous reports The main indication for this substance appears to be generalized Hodgkin's disease, but favourable results may be obtained also in the treatment of reticulosarcomatosis and presumably also in lymphosarcomatosis

The results of Natulan in Hodgkin's disease (assessed on the basis of the number and duration of remissions) appears to be at least as good as that of the best cytostatics so far (chlormethine, cyclophosphamide, vinblastine)

Natulan may be administered by mouth presumably with the same effect as when given intravenously In the future we intend to use oral treatment alone in all cases except where a very rapid effect is decisive Neither in the

literature nor in the present material has there been any instance of cross resistance to previously used cytostatics so that results may presumably be obtained with Natulan even in patients who are resistant to other cytostatic therapy

An important complication of the treatment is bone marrow depression so that the patients should be checked at 1—2 week intervals for the development of leukocytopenia and thrombocytopenia which may appear up to 4 weeks after the discontinuation of the treatment

Apart from nausea, a few severe reactions have been observed partly manifested as skin rash and elevation of temperature and partly as a lowering of blood pressure after intravenous injection of Natulan Therefore, this mode of administration should be started with a low dose

Mention should also be made of the potentiating effect of Natulan upon substances such as alcohol barbiturates phenothiazine and imipramine derivatives so that these substances should be used only with great caution during Natulan therapy

After remission has occurred — usually after administration of 5—6 g intravenously over 12—14 days or of 5—15 g orally over 3—6 weeks — the treatment should probably in most cases be continued by a low oral maintenance dose of 50—150 mg daily

In cases of recurrence an increased or renewed Natulan therapy has been effective in several cases

Like other cytostatics Natulan cannot replace X ray irradiation in localized Hodgkin s disease

Summary

Twenty three patients with malignant systemic diseases and 12 patients with metastasizing solid tumours were treated with a fundamentally new cytostatic (Natulan®) of an unorthodox chemical type

A pronounced effect was obtained in generalized Hodgkin s disease 13 out of 14 treated patients going into complete or partial remission

Furthermore, partial remission was obtained in a few cases of reticulo sarcomatosis and lymphosarcomatosis while only one of 12 patients with metastasizing solid tumours responded to the treatment — and then only for a short time

The value of Natulan therapy in generalized Hodgkin s disease appears to be at least as high as that of the cytostatics used hitherto

Acknowledgements

Two of the patients (cases 13 and 14) were followed in the Medical Department (Heads A Aastrup and H P Ostergaard Kristensen) of the Central Hospital Slagelse Four patients were followed in the Medical Department F (Head L Korsgaard Christensen of the Gentofte Hospital and four were followed in the Radium Centre (Head H Johansen) Copenhagen

We are grateful for the permission to include these patients

We are indebted to Hoffmann LaRoche for a generous supply of Natulan

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From the Cardiovascular Laboratory of Medical Department B (Head A Tybjærg Hansen)
Rigshospitalet (University Hospital) Copenhagen Denmark

Intracardiac Phonocardiography in the Diagnosis of Left Ventricular — Right Atrial Shunt

By

Alf Wennevold

Defects of the ventricular septum with shunting of blood from the left ventricle to the right atrium have been recognized in vivo in increasing numbers during the last years (1—3, 5, 7—13)

The accurate diagnosis was usually established at the operating table or by haemodynamic investigations involving the left side of the heart (2, 8 12) The aim of this paper is to demonstrate that these defects may also be diagnosed by means of a simple right heart catheterization with intracardiac phonocardiography

Anatomy and classification

The communication between the left ventricle and the right atrium may take either of two forms (6 8)

1 The defect of the membranous part of the ventricular septum lies above the orifice of the tricuspid valve and is associated with a defect in the floor of the right atrium (type I fig 1)

2 The ventricular septal defect is associated with tricuspid insufficiency so that arterialized blood is shunted either to the right ventricle with regurgitation of some of the blood to the right atrium type II A fig 1) or directly to the right atrium, if the edges of the opening in the septal leaflet of the tricuspid valve are fused to the right side of the margins of the ventricular septal defect (type II B fig 1) Type II is the most common form

The atrial septum is intact and atrioventricular defects are consequently not included in the definition of left ventricular — right atrial shunt

Methods

The Allard Laurens micromanometer has been used to record pressure and intracardiac phonocardiogram (14 The arteriovenous shunt to the right atrium was demonstrated by oxygen determinations and or with the platinum electrode catheter with hydrogen inhalation

Diagnosis

When during systole a jet of blood passes from the left ventricle through the ventricular septal defect a murmur is produced in the defect and is transmitted with the blood flow The registration of this systolic murmur

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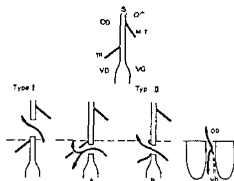


Fig 1 Diagrammatic representation of communication between the left ventricle and the right atrium S = septum OG = left atrium OD = right atrium MIT = mitral valve TR = tricuspid valve VG = left ventricle VD = right ventricle From Laurichesse et al Arch Mal Cœur 57 703 1964

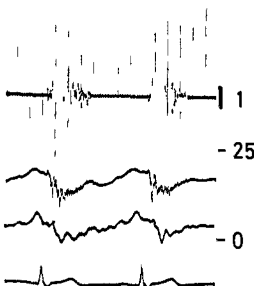


Fig 2 Intracardiac phonocardiogram (upper tracing) showing holosystolic murmur in the right atrium (case no 4511) In the upper right margin the calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line Pressure is recorded both with the micromanometer at the tip of the catheter (middle tracing) synchronously with the sound and through the sidehole (lower tracing) 1 5 cm from the tip the latter tracing being calibrated to 0 and 25 mm Hg

by the micromanometer situated in the right atrium establishes the diagnosis (2, 4 8) (fig 2)

The murmur is usually confined to a restricted area in the right atrium i e above and close to the tricuspid valve If the murmur also is recorded in the right ventricle (fig 3) a type II A defect is present (fig 1)

The differential diagnosis includes atrial septal defects of the ostium primum type and atrio ventricular defects both with tricuspid insufficiency These conditions are ruled out by the failure of passing the catheter to the left atrium in spite of careful search of the atrial septum Rupture of an aneurysm of the sinus of Valsalva to the right atrium and a fistula of a coronary artery to the right atrium are both accompained by a continuous murmur and are easily ruled out

The systolic murmur of aortic stenosis and of coarctation of the right pulmonary artery may be recorded in the right atrium (15) but the murmur is rather faint and of the ejection type and no shunt is found

Materials and results

From December 1963 to November 1965 the diagnosis of left ventricular — right atrial shunt was established in nine patients by intracardiac phonocardiography during right heart catheterization (table I) In one patient (case no 5730) a patent ductus had previously been closed in all others no complicating heart disease was present

The defect was probably of type II A (fig 1) in all cases, as the systolic murmur was also recorded in the right ventricle (fig 3)

In one patient (case no 5638) the catheter tip passed from the right atrium directly to the left ventricle (fig 4) and in another patient (case no 1191) the diagnosis was confirmed at operation

At external auscultation a medium to high pitched holosystolic murmur was heard in all patients (fig 5) it was grade 4—5 (of 6) in all but one in whom grade 3 was

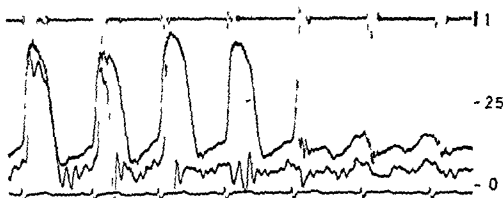


Fig 3 Intracardiac phonocardiogram on withdrawal of the catheter from the inflow tract of the right ventricle to the right atrium (case no 4394)

TABLE I Findings in nine patients with left ventricular—right atrial shunts

Case no	Age (yrs)	Difference of oxygenation in saturation %: c v to low in r a	Difference of oxygenation in saturation %: low in r a to p a	Flow rate P/S	H used	Pressure in p a (mm Hg)	Systolic murmur in: r a (mm Hg)	Systolic murmur in: r v (mm Hg)
4394	12	6	8	2 4		33/10	3 0	3 0
4511	22	2	3	1 2	+	20/9	2 0	3 5
4984	16	4	4	1 6	+	25/8	2 0	2 0
1191	19	4	14	2 5		37/12	1 8	2 5
5638	17	2	9	1 7	+	23/13	2 5	2 5
7036	20	7	3	1 6		27/16	0 5	2 5
5496	14	2	4	1 3	+	29/11	5 0	5 0
7212	50	14	2	*1 5		81/26	3 0	0 8
5730	15	3	3	1 3	+	42/11	2 5	2 5

* A right-to-left shunt was also present

c v average of superior and inferior caval veins, r a = right atrium p a pulmonary artery
P/S pulmonary blood flow/systemic blood flow H = hydrogen electrode, r v right ventricle

heard (case no 4511) it was maximal in the third of fourth left intercostal space at the sternal border

The electrocardiogram showed incomplete right bundle branch block in six patients while it was within normal limits in the remaining three patients (case nos 4511 4984 and 5638) The chest roentgenogram was considered normal in one patient (case no 5496) and in the other eight patients it showed varying abnormalities from prominence of the pulmonary artery to augmented heart with increased vascularization of the lung vessels

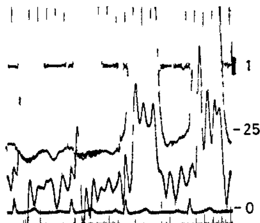


Fig 4 Intracardiac phonocardiogram while the tip of the catheter is passed from the right atrium to the left ventricle (middle tracing) while the sidehole (lower tracing) is pushed from the right atrium to the right ventricle (case no 5638)

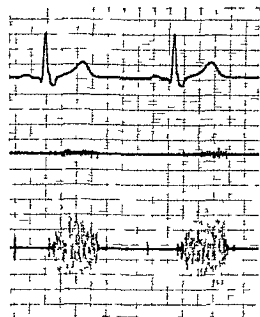


Fig 5 External phonocardiogram from the fourth left intercostal space (case no 4511) (Mingograph 31 B Elema Schonander)

Discussion

In patients with the clinical findings of a ventricular septal defect the diagnosis of left ventricular — right atrial shunt should be suspected, when during right heart catheterization an arterio venous shunt to the right atrium is found without it being possible to pass the catheter tip *through an atrial septal defect to the* left atrium (2, 11)

In a few cases the catheter passes directly from the right atrium to the left ventricle (1, 9, 11) Otherwise a positive diagnosis may be established by selective angiocardiography with injection into the left ventricle (2, 8, 12), *but the diagnosis may equally well be* made by the simpler method of right heart catheterization with intracardiac phonocardiography, this was also shown previously by others (1, 4, 8)

Summary

The diagnosis of left ventricular — right atrial shunt is possible by a simple right heart catheterization with intra cardiac phonocardiography

The diagnosis was established in nine patients, whose clinical and haemodyna mic findings are briefly presented

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From the Department of Medicine (Head P Brummer M D), University of Turku
Turku Finland

Early Ambulation in the Treatment of Myocardial Infarction

By

PEKKA BRUMMER VEIKKO KALLIO and EERO TALA

In the treatment of myocardial infarction we have since the summer of 1952 in this hospital, followed the principle of permitting the patient to sit up in bed about one week after the infarction if the acute symptoms have passed Bed side toilet privileges have been allowed from the beginning In the absence of specific contra indications ambulation has been permitted from a fortnight after infarction Recently the period of confinement to bed has been shortened further being now only about 10 days on the average In other respects the treatment has been the usual

In 1956 (1) we published our results of treatment in the period July 1, 1952—December 31, 1954 (series I) The results were very good during ambula tion in the hospital but during the first month after discharge there was a relatively high incidence of relapse (8 2 %) Since in those years anticoagulant therapy was discontinued when the patient was discharged from the hospital we considered that this high incidence may have been due either to early ambulation or to discontinuance of anticoagulant therapy As a control we therefore studied our cases of myocardial infarction in the period July 1, 1957, to December 31, 1959 (series II) (2) Thera peutically this series differed from the first one merely in the respect that the anticoagulant therapy was continued after the patients were discharged from the hospital The results of treatment during hospitalization were again very good Furthermore the incidence of recurrences during the first month after discharge had dropped almost to one fourth of that in the first series being now only 2 5 %

Material

Despite the good results demonstrated by these series early ambulation in the treat ment of myocardial infarction has not gained the wide use which in our opinion it deserves We have therefore supplemented our earlier results by studying a third series from the period July 1 1962— December 31

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TABLE I Number of patients mean age sex distribution mean length of bed rest and hospitalization and mortality during bed rest

	Series I 1 7 1952– 31 12 1954	Series II 1 7 1957– 31 12 1959	Series III 1 7 1962– 31 12 1964
No of patients	332	565	775
Mean age (years)	57	58	59
Males (%)	71 3	81 9	74 4
Average length of bed rest (days)	16 2	12 0	10 3
Average length of hospitalization (days)	22 6	20 1	18 9
Deaths during bed rest (%)	22 4	22 5	27 9

TABLE II Follow up series and results

	Series I 1 7 1952– 31 12 1954	Series II 1 7 1957– 31 12 1959	Series III 1 7 1962– 31 12 1964	
No of patients	236	321	559	(396)[1]
Pathologic index rate				
0—39	60 5%	65 4%	69 6%	
40—79	32 9%	27 4%	30 0 %	
80—	6 6%	7 2%	5 4%	
Type of infarct				
Anterior anterolateral or anteroseptal	60 0%	60 2%	46 9%	
Posterior or posterolateral	29 0%	33 3%	39 4%	
Purely lateral	1 9%	2 2%	4 5%	
Purely septal	3 8%	0 6%	1 9%	
Uncertain	5 3%	3 7%	7 3%	
Complications during ambulation in hospital				
Sudden death	2	1	11	(8)
Recidive myocardial infarction	1	2	5	(4)
Congestive heart failure	5	10	7	(3)
Complications during 1st month after discharge from the hospital				
Sudden death	1	—	5	(3)
Recidive myocardial infarction	21	8	6	(4)
Rehospitalized because of chest pain	8	8	7	(6)

[1] Patients receiving anticoagulant therapy within brackets

1964 (series III) These three series and the results obtained in each of them are presented in tables I and II Each series includes only clinically confirmed cases of myocardial infarction treated in our hospital during the period stated

As is seen in table I the three series are comparable with respect to the mean age and the sex distribution of the patients Although the series cover periods of equal length the number of cases has more than doubled during the whole 10 years Since there has been no noteworthy change in the grade of severity of the cases the increase evidently is not a result of improved diagnosis Nor is an explanation provided by the increased population and its higher mean age Consequently there appears to be a true increase in the incidence of myocardial infarction in the Turku area during the period concerned

Results

The complications during ambulation in hospital and during the first month after discharge from hospital are presented in table II which also shows the grade of severity (pathologic index rate of Schnur (3)) and the location of the infarcts The total patient numbers stated are the number examined at follow up In series I a follow up examination was made of 91 5 % and in series III of 100 % of the patients alive at the time ambulation was begun In the compilation of series II only the patients on continued anticoagulant therapy were included as was mentioned above, and 73 3 % were therefore followed up In connecting series III we also compared the patients with and without anticoagulant therapy the former group being shown in the table in brackets It is seen from these figures that there was no apparent difference in the incidence of complications, between the group with and that without anticoagulant therapy The omission or discontinuation of anticoagulants was indicated not by the severity of the infarction but by complicating diseases or poor laboratory control possibilities after the patient s discharge from hospital On the basis of these results it therefore appears probable that the incidence of complications in connection with early ambulation is not influenced to any noteworthy extent by whether anticoagulant therapy was given or not, whereas series I gives evidence suggesting that the discontinuation of anticoagulant therapy when the patient leaves the hospital increases the danger of recurrence of infarction during the first month after discharge

In series III there was a somewhat higher incidence of sudden deaths during ambulation in hospital than in the previous series but no increase was seen in the incidence of recurrence of infarction The average duration of confinement to bed among the patients dying during ambulation was the same as that in the total series In considering the mortality one must keep in mind the reduced length of bed rest in view of which some of the deaths which now took place during ambulation would in the earlier series have occurred during confinement to bed and appeared in this group in the statistics Of the patients dying suddenly during ambulation one had recurrence of infarction two had diabetes two chronic renal disease and one severe peripheral arteriosclerosis Accordingly only the remaining five patients who died suddenly had an

"uncomplicated' infarction The mortality during ambulation can still be regarded as very low and by no means higher than it would have been during a longer confinement to bed

The number of complications during the first month after discharge continued to be low in series III

Autopsy was performed on six of the patients who died suddenly Cardiac rupture or pulmonary embolism was not found in any of these patients

No case of severe thrombo embolic complication was diagnosed in these three series There is a possibility, however, that some sudden deaths may have been due to pulmonary embolism

Discussion

From the results presented it is evident that early ambulation during the treatment of myocardial infarction does not constitute an increased danger to the patient, for example, there appears to be no noteworthy risk of cardiac rupture or aneurysm Autopsy on patients in series III who died during ambulation did not disclose cardiac rupture in any of the cases During the collection of series I, 48 patients had a follow up X ray examination 6 months after the myocardial infarction and only one cardiac aneurysm was found

The advantages of early ambulation are obvious 1 a reduced risk of thromboembolic complications, 2 a more rapid physical and psychical recovery of the patient, and 3 a shorter period of hospitalization and thus a reduced load on hospital beds

Summary

In 1952 we began in our hospital to restrict to two weeks the length of confinement to bed of patients with myocardial infarction Gradually the length of bed rest has decreased further and is at present 10 3 days on the average We present the results in a series of patients treated in 1962—64 and make a comparison with previously published series from 1952—54 and 1957—59 from the same hospital

The results show that early ambulation is not associated with any increased danger to the patient, while it obviously entails advantages in preventing thrombo embolic complications and maintaining the physical and psychical fitness of the patient Furthermore the length of hospitalization is reduced by early ambulation

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From the Departments of Medicine and Medical Chemistry, University of Turku Turku, Finland

Serum and Urinary Urobilinoids in Hepatic Diseases

By

AAPO LEHTONEN, VEIKKO NÄNTO and PEKKA BRUMMER

Increased urinary excretion of urobilinoids has long been considered a sensitive indicator of parenchymatous liver disease, but determination of serum urobilinoids has not yet had clinical significance in spite of several applications of the analytical methods (3, 9) Lozzio et al (4, 6) have used a method based on Schlesinger's reaction to determine the serum urobilinoids, and they have also investigated different urobilinoids (d i and l urobilins) in serum and urine by electrophoresis (5) With the methods of Lozzio et al, we have analyzed the serum and urinary urobilinoids in healthy subjects and in patients with hepatic and biliary lesions to find out the diagnostic significance of these methods

Methods

The determination of urobilinoids in serum was made according to Lozzio et al (6) The non hemolysed blood sample was centrifuged at +4 C Two ml of absolute ethanol 2 ml of 20 % solution of ferric chloride 10 ml of distilled water and 4 ml of 20 % NH_4OH were added to 2 ml of the serum. After centrifugation the supernatant was filtered if necessary and 0 05 ml of 50 % solution of glacial acetic acid and 4 ml of Schlesinger's reagent were added to 4 ml of filtrate Absolute ethanol was used instead of Schlesinger's reagent as the blank A 0 25 % solution of trypaflavine was used as a standard and the fluorometric measurements were made with the fluorescence accessory of a Beckman DU spectrophotometer 0 330 μg of trypaflavine corresponded to 0 15 μg of urobilinogen (Bios Laboratories Inc New York) All urobilin determinations were performed on fresh specimens because the urobilinoid content of the specimen decreases on standing

The electrophoretic fractionation of urobilinoids in urine was performed according to Lozzio et al (5) The urobilinoids were extracted by the following procedure from urine 10 ml of 20 % ferric chloride and 20 ml of 20 % NH_4OH were added to 20 ml of urine After centrifugation the solution was acidified with 3 N hydrochloric acid to pH 1—2 and extraction with chloroform was carried out The separation of the urobilins was made by electrophoresis on Whatman No 3 MM paper with use of pyridine acetate buffer pH 5 9 (175 ml pyridine 25 ml glacial acetic acid and distilled water to 5 000 ml) for 4—5 hr at 50 V/cm After the electrophoresis the paper was dipped in Schlesinger's reagent and examined under ultraviolet light

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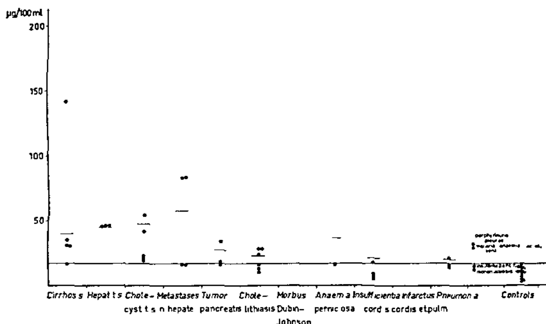


Fig 1 The distribution of serum urobilinoid values in different pathological states and in the group of healthy control persons

at 325 mμ The fractions were identified according to Lozzio et al (5) and the intensity of fluorescence was estimated with an arbitrary scale (0 + ++ +++)

Material

The serum urobilinoids of 15 normal individuals and of 80 patients (52 suffering from different hepatic and biliary disorders and 28 from miscellaneous diseases) were studied Diagnosis was based on clinical status laboratory findings X ray examinations and in several cases on liver biopsy and operative findings In all cases the diagnosis was clinically confirmed

Results

The results of the quantitative determination of serum urobilinoids are shown in fig 1 In the healthy test subjects the level of serum urobilinoids was 10 ± 7 µg/100 ml The highest concentrations of urobilinoids were found in hepatic cirrhosis, in hepatitis and in pernicious anemia In metastatic cancer of liver and in cholecystitis there was also an increased amount of serum urobilinoids In biliary obstruction without clinical cholecystitis the values for serum urobilinoids were normal or slightly elevated In myocardial infarction and in pneumonia there was a slight increase over the normal level but the values were generally normal in cardiac incompensation In one case of hepatitis the concentrations of serum urobilinoids and bilirubin were followed during the disease (fig 2) The level of serum urobilinoids compared with other laboratory investigations (bilirubin alkaline phosphatase and glutamic-oxalacetic transaminase) is shown in fig 3

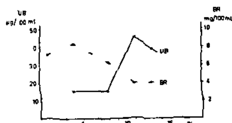


Fig 2 The changing pattern of serum urobilinoid and bilirubin concentrations in a patient during his recovery from hepatitis

Electrophoretic separation of urinary urobilinoids of normal subjects gave nearly always two fractions, identified as l and i urobilins, but d urobilin was not found In hepatic diseases and in several other cases in which the level of serum urobilinoids were increased the urine also contained d urobilin (fig 4) Especially strong fluorescence of d urobilin was found in some cases of hepatitis, of metastatic cancer of liver and of pernicious anemia and in one case of cholecystitis L-urobilin was absent in 5 and i urobilin in 7 patients with hepatic disease

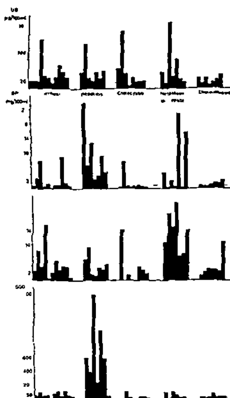


Fig 3 The level of serum urobilinoids in patients with hepatic and biliary diseases compared with corresponding values for serum bilirubin alkaline phosphatase and glutamic oxalacetic transaminase

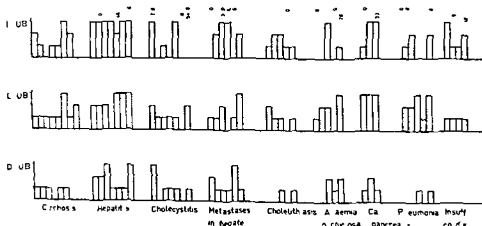


Fig 4 The proportional distribution of electrophoretically separated urinary urobilinoid fractions in different diseases Evaluation 0 + ++ +++ At the top of every column the simultaneous concentration of urobilinoids in serum is given

Discussion

The concentration of serum urobilinoids was noticeably increased in hepatitis, cirrhosis and cholecystitis and also in metastatic cancer of liver and pernicious anemia In hepatitis the serum concentration of urobilinoids depended on the stage of the disease, reflecting obviously the state of the intrahepatic cholestasis The values for serum urobilinoids increased when the bilirubin content began to decrease (fig 2) No other significant correlation was found between urobilinoid level and the liver function tests performed (fig 3)

Obviously the quantitative determination of serum urobilinoids does not particularly assist in differential diagnosis of liver diseases It can perhaps provide information concerning the stage of any hepatocellular lesion and the state of recovery from e g hepatitis On the other hand however, hyperurobilinaemia appears in cholecystitis, in which there is virtually no general parenchymatous lesion (11)

According to Lozzio and Royer (7), normal plasma and urine contain only i urobilin In our material l and i urobilin were found quite regularly in the urine of normal individuals and of patients with hepatic and other diseases This is in agreement with the results of Watson (12) Baumgartel (1, 2) Rudolf (10) and Maier and Schwarz (8) have published data which show that in the presence of liver injury i urobilin is the most elevated fraction in the urine and that in normal subjects and in patients with hemolytic anemia l urobilin is preponderant According to our results there is very little d urobilin or none at all in the urine of healthy individuals, but in most diseases with elevated serum urobilinoids this fraction was also increased The urinary excretion of different urobilinoids could not be correlated with the hepatic or other disease in our material

Summary

The level of serum urobilinoids in healthy subjects and in patients with hepatic and biliary diseases, was determined by the fluorometric method according to Lozzio et al The urinary excretion of urobilinoids was studied by electrophoresis The elevated values for serum urobilinoids were found in a variety of hepatic and biliary diseases, obviously without greater differential diagnostic significance In the urine of normal subjects l- and i urobilin were almost regularly found, and in hepatic, biliary and some other diseases with elevated serum urobilinoids the urine also contained d urobilin

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TABLE I Statistical analysis of the serum iron values of the total number of patients (60) after oral iron given with a standard meal (skimmed milk powder) with or without ascorbic acid or alcohol (ethanol)

	Mean difference between fasting and maximum serum iron	Difference calculated	Mean diff	S D	t test	P
A On standard meal	$\bar{x}_1$ = 61 11	$\bar{x}_2-\bar{x}_1$	40 15	74 27	4 15	2% **
B On standard meal + ascorbic acid	$\bar{x}_2$ − 101 26	$\bar{x}_3-\bar{x}_1$	40 36	64 97	4 77	2% **
C On standard meal + alcohol	$\bar{x}_3$ − 101 48					

TABLE II Statistical analysis of the serum iron values in those patients (46) who showed increased serum iron after oral iron given with a standard meal (skimmed milk powder) with or without ascorbic acid or alcohol

	Mean difference between fasting and maximum serum iron	Difference calculated	Mean diff	S D	t test	P
A On standard meal	$\bar{x}_1$ 78 13	$\bar{x}_2-\bar{x}_1$	52 56	80 7	4 36	1% *
B On standard meal + ascorbic acid	$\bar{x}_2$ 130 69	$\bar{x}_3-\bar{x}_1$	52 30	69 8	5 03	1% **
C On standard meal + alcohol	$\bar{x}_3$ 130 43					

all cases by direct fasting aspiration eventually after stimulation by histamine In 26 cases achylia was present

Methods

Fasting patients were given 300 mg ferrous iron as ferro-fumarate together with 100 g skimmed milk powder on 3 successive days One day the iron was given without any further addition (called A day) another day together with 250 mg ascorbic acid (called B day) and on the third day together with 12 g alcohol (as French brandy) (called C day) The patients got the test doses in one of the randomly chosen sequences of A B and C days (ABC ACB BAC BCA CAB CBA) Serum iron was estimated before the iron doses were given and repeated after 2 4 and 6 hours The serum iron estimations were performed by the method of Ramsay (2) with modifications The difference between the fasting serum iron and the maximum level reached within the six hour experimental period has been taken as a measure of the iron absorption

Results

In 14 patients there was no detectable increase in serum iron following the iron doses In none of these patients was the difference between the maximum and the fasting serum iron value above 17 microgram % on any day In accordance with earlier results (3) these data suggest that no iron absorption has taken place Accordingly it seemed logical to analyse the material on the following lines

1 Analyse the total number of patients (60)

2 Analyse all patients who responded to the administered iron doses with increased serum iron values (46),

3 Analyse that group of patients where no increase in serum iron could be detected (14)

The results of statistical analysis of the serum iron values of *the total number of patients* are demonstrated in table I and of *all the patients who responded to the iron doses* in table II The data in table I and II are based upon the differences between the maximum and the fasting serum iron values on each day, and variations in these differences are considered to be an effect achieved by the dosage of alcohol or ascorbic acid Table I and II demonstrate a significantly increasing effect on the iron absorption both of alcohol and of ascorbic acid In spite of the relatively large standard deviations shown in the tables the t test shows P values of 2 and 1 per cent respectively A comparison between alcohol and ascorbic acid shows no difference in effect

Of the last 14 patients mentioned above 9 had acute or chronic infectious disease, 2 had collagen disease , 1 was a chronic alcoholic with gastritis and 1 had a wide spread gastric carcinoma The ESR varied between 18 and 140 mm/hour, and the results of the estimations of gastric acidity of this group of patients were almost entirely matched those for the total group of patients

Discussion

Published experiments on anaemic rats (4) have shown that alcohol and ascorbic acid can increase the absorption of radioactive iron, their effects being equivalent The results presented in this paper demonstrate that alcohol and ascorbic acid have a similar effect on the absorption of ferrous iron in patients with iron deficiency The subjects studied were all hospital patients with various diseases in addition to iron deficiency This has made the material very heterogeneous and may on top of individual differences explain the great variations in the results from one person to an other In spite of this, the results obtained seem to be convincing Alcohol does indeed increase the absorption of ferrous iron in iron deficient subjects and this effect is not dependent on extremely high doses of iron or on the presence of any liver damage The measured effect seems to be equal to that of ascorbic acid

Summary

Iron absorption has been studied by means of oral doses of ferrous iron The iron has been given together with a standard meal with or without addition

of alcohol or ascorbic acid to 60 patients with iron deficiency Based upon analyses of serum iron, the results show that both alcohol and ascorbic acid significantly increase iron absorption, their effects being equivalent

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From Medical Department A (Head K Brochner Mortensen M D) and the Chromosome Laboratory Department of Obstetrics and Gynaecology A—I (Head D Trolle M D) Rigshospitalet Copenhagen Denmark

Chromosomal Findings in Two Cases of Acute Erythro-leukaemia

By

MOGENS KROGH JENSEN

The recent development of suitable techniques for chromosome studies (14 18) has aroused considerable interest in the cytogenetical study of many haematological diseases So far a specific chromosome aberration has been demonstrated only in chronic myelocytic leukaemia (2 16) Many other diseases have not yet been adequately studied due in part to their rare occurrence At the present time, therefore the possibility that specific chromosomal aberrations exist in other haematological disorders cannot be dismissed The present report deals with the chromosomal findings in two cases of Di Guglielmo s syndrome (acute erythro-leukaemia)

Case reports

Case I 75 year old housewife At the age of 50 she had several series of radiotherapy (dose unknown) towards the spine because of spondylitis At the beginning of May 1965 she was admitted to this department after having suffered from increasing fatigue for the last six months The tongue was devoid of papillae Hb 4 1 g % Leucocytes 1 200 /µl with 10 % "blast cells 1 % myelocytes 48 % neutrophils 23 % lymphocytes 2 % monocytes 16 % reticulum cells Three erythroblasts per 100 nucleated cells were found in the peripheral blood Thrombocytes 35 000/µl Hydrochloric acid was present in the gastric juice Serum lactic dehydrogenase 48 units/µl (normal value 7—23 units/µl) Serum vitamin B_{12} 116 µg/ml. Serum folic acid 0 007 µg/ml (lactobacillus casei assay normal value 0 004—0 018 µg/ml) The Schilling test was normal The bone marrow was completely dominated by megaloblasts several of which had two or three nuclei In addition several forms of reticulum cells and some blast cells were seen During treatment with vitamin B_{12} some restoration of the papillae of the tongue was seen A marked reticulocytosis occurred but the red-cell count remained unchanged The marrow was still dominated by erythrocytic precursors which were much less megaloblastic than those of the first marrow aspirate The marrow was locally completely dominated by macronormoblasts A number of "blast cells together with some cells with neutrophilic granules of the cytoplasm which should be interpreted as myelocytes, were found The patient was given several

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transfusions of packed red cells before discharge

She was readmitted in the middle of July, 1965 with haematemesis and melaena Hb 6 0 g % Thrombocytes 34 000—5,000/μl The white cell count was increasing (4 600—46,000/μl) with about 80 % myeloblasts The bone marrow was dominated by myeloblasts and there was a conspicuous absence of erythropoietic cells Death occurred at the beginning of August 1965 At autopsy the bone marrow liver spleen and lymph nodes were found infiltrated by immature mononuclear cells

Case 2 62 year-old knitter Because of recurrent abscesses of her left armpit the patient had had a course of radiotherapy (800 r) five years ago At the end of June, 1965 the patient was admitted to this department after having suffered from fatigue and increasing dyspnoea for six months Numerous petechiae were seen on the skin of the extremities Hb 5 5 g % Leucocytes 18 200/μl with 50 % blast cells most of which had an irregular nucleus Some of the immature cells were monocytoid in appearance Three erythroblasts per 100 nucleated cells were found in the peripheral blood Thrombocytes 20 000/μl Serum B_1 1 128 μg ml Serum folic acid 0 007 μg/ml (lactobacillus casei assay normal value 0 004—0 018 μg/ml The marrow was dominated by immature monocytoid cells and megaloblasts The patient was treated with transfusions of packed red cells

She was readmitted at the end of July 1965 on account of increasing stridor *Hb 10 1 g % Leucocytes 7 000 μl* with 68 % immature cells most of which were monocytoid Thrombocytes 46 000/μl The marrow was dominated by blast cells myelocytes and monocytoid cells Treatment with 6-mercaptopurine was instituted but the condition rapidly deteriorated and death occurred at the beginning of September 1965 At autopsy the bone marrow liver spleen kidneys tonsils and mucosa of trachea were found infiltrated by immature mononuclear cells

Material and methods

Chromosome analysis was performed on bone marrow aspirates and on cells from the peripheral blood from both patients In addition metaphases from a skin biopsy of the first patient were analyzed

The marrow specimens were treated according to the technique described by Tjio and Whang (18) Cells from the peripheral blood were cultured for 72 hours according to a slight modification of the method of Moorhead et al (14) In some cultures phytohaemagglutinin was not added These cultures were harvested after 48 hours The skin biopsy was cultured according to a modification of the method of Harnden (7)

Results

Case 1 Marrow was sampled on three occasions before any cytotoxic treatment had been given The leukocytes of the blood were cultured twice The marrow aspirates and the blood cultures without phytohaemagglutinin added were characterized by three stem lines, two of which contained marker chromosomes, viz a ring chromosome which had the size of one of the minor chromosomes of group 6 x 12 (fig 1 a and 1 b) and a ring chromosome of the size of a chromosome of group 19—20 (fig 2), respectively The third stem line had no marker chromosome Two metaphases containing both marker chromosomes were found A mode of 42 chromosomes was found In cells with a marker chromosome the hypoploidy was due to one chromosome missing in group 13—15 and two chromosomes missing in the groups 16—18 and 19—20, respectively In metaphases without a marker chromosome there was found either an extra chromosome in group

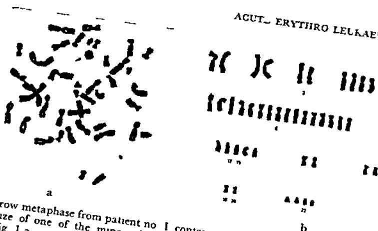


Fig 1 a Marrow metaphase from patient no 1 containing 42 chromosomes with a ring chromosome of the size of one of the minor chromosomes of group C b Karyotype of the marrow metaphase of fig 1 a

6 x 12 or only one chromosome missing in group 19—20 In nearly all the metaphases a minute acentric fragment was present In some of the marrow and blood preparations polyploid cells were found the majority of these contained two marker chromosomes In all metaphases the chromatids appeared blurred and irregular

As seen from table I a characteristic feature of the marrows and the blood specimens cultured without phytohaemagglutinin was a change in the relative proportions of the three stem lines with time

In the first blood specimen cultured with phytohaemagglutinin for 72 hours a sharp mode of 46 chromosomes with a normal female karyotype was found The abnormal karyotypes present in the marrow cells were not seen

In the second blood specimen cultured with phytohaemagglutinin two stem lines were seen viz metaphases with a normal female karyotype and metaphases with 42 chromosomes all containing a ring chromosome of the size of one of the minor chromosomes of group 6 x 12

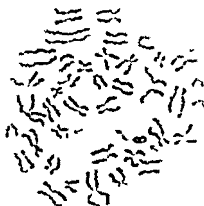


Fig 2 Marrow metaphase from patient no 1 containing 42 chromosomes with a ring chromosome of the size of a chromosome of group F together with a minute acentric fragment

The metaphases of the skin biopsy were characterized by a normal female karyotype However numerous polyploid cells without marker chromosomes were seen

TABLE I Distribution (%) for patient no 1 of the various stem lines in marrow and blood cultured without phytohaemagglutinin and percentage of mitoses belonging to the erythrocytic and granulocytic precursors

Date	Type of tissue	A-cells	B-cells	C-cells	D-cells	Mitoses belonging to e p	g p
10–5–65	Marrow	7	29	14	0	84	6
29–5–65	Blood without pha	2	11	8	0	—	—
9–6–65	Marrow	26	13	10	1	96	4
31–7–65	Marrow	34	9	6	1	0	100
31–7–65	Blood with out pha	34	3	13	0	—	—

A cells cells with a ring chromosome of the size of one of the minor chromosomes of group C
B-cells cells with a ring chromosome of the size of one of the chromosomes of group F
C-cells cells without a marker chromosome
D cells cells with both marker chromosomes

pha phytohaemagglutinin
e p erythrocytic precursors
g p granulocytic precursors

TABLE II Chromosomal findings in two cases of erythro-leukaemia

Case no	Date	Type of tissue	Total cells counted no	No of chromosomes <40	40	41	42	43	44
1	10 5 65	Marrow	50	2		6	36	3	1
	29 5 65	Blood without pha	21			1	18		
	29 5 65	Blood with pha	50	1	2	1	1	2	
	9 6 65	Marrow	50		4	5	37	4	
	15 6 65	Skin	50				1		
	31 7 65	Marrow	50	2	3	1	39	4	1
	31 7 65	Blood without pha	50	5	3	6	31	5	
	31 7 65	Blood with pha	50			2	23	2	1
2	23 6 65	Marrow	50				1		
	23 6 65	Blood with pha	50						1
	19 8 65	Marrow	50						
	31 8 65	Marrow	50					1	1

pha phytohaemagglutinin

Case 2 Two marrow aspirates were obtained before treatment with cytotoxics had been given Therapy with 6-mercaptopurine was instituted 12 days prior to aspiration of the third marrow Blood cultures were set up twice with and without phytohaemagglutinin One of the cultures with phytohaemagglutinin added was unsuccessful and the cultures without phytohaemagglutinin yielded no dividing cells The modes of the marrows and the blood specimen were diploid with a normal karyotype In no instance did the chromatids have the blurred appearance usually seen in acute leukaemia In the third marrow aspirate structural abnormalities were shown by 18 % of the cells as compared with 0 and 4 % of the cells of the first and the second marrow respectively The results are summarized in table II

Discussion

Reports of chromosome investigations in erythro leukaemia are scarce Two of the patients in the series of Fitzgerald et al (5) had erythro-leukaemia The marrow metaphases of the first patient had a normal female karyotype, whereas the second patient s marrow had a mode of 45 chromosomes with one chromosome of group 6-x 12 missing One of the chromosomes of group 16—18 was abnormally short McClure et al (13) found a sharp mode of 45 chromosomes, with one chromosome of group 6 x 12 missing in the marrow of their patient Cultured cells from the peripheral blood showed a normal male karyotype Di Grado et al (6) found a ring chromosome with the size of a A 2 chromosome in 90 per cent of the marrow metaphases from a patient with acute

45	46	47	48	49	50	>50	Polyploid cells no	Cells with structural abnormalities (%)
1							7	100
	2						0	90
4	37	1				1	1	12
							1	96
7	41	1					7	2
							0	96
							0	94
1	21						0	58
3	45					1	0	0
1	47			1			0	4
3	47						0	4
2	44	2					0	18

erythro-leukaemia The mode was 45 chromosomes with no chromosome consistently missing Recently, Kiossoglou et al (9) published a report of the chromosomal findings in 65 patients with acute leukaemia including 16 patients with Di Guglielmo s syndrome Seven of the latter patients showed no grossly visible chromosomal changes, whereas in 9 patients a varying percentage of the marrow metaphases showed abnormal karyotypes The chromosome mode was aneuploid only in 2 cases The aneuploidy was due to trisomy of group 6 x 12 and group 19—20, respectively In another patient the Ph[1] chromosome was present in all metaphases analyzed Heath and Moloney (8) found two major cell lines of 39 chromosomes associated with chromosomal breakage, polyploidy and endoreduplication in two marrow aspirates from a patient with Di Guglielmo s syndrome Thus the chromosomal findings in erythro leukaemia present a wide spectrum as found in other types of acute leukaemia Nine of the 23 cases recorded — including the present case 2 — have shown a normal chromosome constitution No specific chromosome aberration common to the cases with abnormal findings has been demonstrated

In chronic myelocytic leukaemia the characteristic chromosomal abnormality (the Ph[1]-chromosome) is present not only in the granulocyte precursors but also in erythroblasts and probably also in megakaryocytes (20 21 22) In contrast it has not yet been resolved whether the red-cell precursors and megakaryocytes in *acute* leukaemia are derived from the same stem cell as the blast cells of the white-cell series In case I of the present study the cellular composition of the bone marrow changed markedly during the study the first marrow aspirate was dominated by megaloblastic erythropoiesis, whereas the second aspirate was considerably less megaloblastic and was locally completely dominated by macronormoblasts The third marrow aspirate consisted almost entirely of myeloblasts All the metaphases examined from the various marrow aspirates obtained during the course of the disease were abnormal An interesting feature of the chromosomal findings in the marrow aspirates is the presence of three clones of cells and the *change in their relative proportion with time* yet with persistence in the last marrow aspirate of some cells belonging to the originally dominating stem line The increasing dominance of the karyotype with a ring chromosome with the size of one of the minor chromosomes of group 6 x-12 suggests that this clone possessed a selective proliferative advantage over the other abnormal clones When the change in relative proportion of the stem lines is compared to the percentage of mitoses belonging to the erythrocytic and granulocytic precursors of the marrow aspirates (table I), it is seen that none of the abnormal clones seems to be specific to any type of marrow cell

Thus the above findings strongly suggest that the erythrocytic and granulocytic precursors had the same abnormal karyotype and hence were derived from a common stem-cell No mitoses of megakaryocytes were seen in the marrow aspirates

The lymphocytes of the peripheral blood cultured with phytohaemagglutinin possessed a normal karyotype, which is evidence that these lymphocytes were cytogenetically unrelated to the leukaemic cells The clone of cells with 12 chromosomes found in the second blood specimen cultured with phytohaemagglutinin is probably attributable to the capacity of leukaemic cells to divide in vitro Cells from a non haemopoietic tissue (skin) had a normal chromosomal constitution apart from a marked polyploidy, which is not usually seen with this technique

Ring chromosomes have been demonstrated in man in association with acute leukaemia (1) other types of neoplasia (11), ionizing radiation (19) cytotoxic treatment (15) and congenital malformations (12) The ring chromosome probably arises when terminal deletions of a chromosome are followed by fusion of the chromatids

In view of the gross chromosome aberrations present in some cases of acute leukaemia it is a puzzling problem that in other cases no visible chromosome aberrations are seen, e g in case 2 of the present report Characteristic clinical differences between cases with normal and abnormal karyotypes have not been demonstrated However, the presence of a normal karyotype obviously does not exclude chromosomal changes not demonstrable by light microscopy

Structural abnormalities of the chromosomes are a common finding in treated (4) as well as in untreated (8) cases of acute leukaemia The structural chromosomal abnormalities found in the two cases of the present report probably differ in aetiology In the first patient the abnormalities were likely to be intrinsic to the leukaemic cells since the patient had not received any cytotoxic treatment before any of the three marrow aspirations Although it cannot be excluded that the structural abnormalities may have been induced by the course of radiotherapy towards the spine given 25 years ago, this possibility seems unlikely in view of the constancy of the abnormalities (viz minute acentric fragments and ring chromosomes) and of the period elapsed since the radiotherapy was given

In the second case the structural chromosomal abnormalities of the last marrow aspirate were probably produced by 6-mercaptopurine which had been started 12 days before That 6 mercaptopurine and its derivative, azathioprine, may produce structural chromosomal abnormalities has recently been demonstrated (3 10 17)

Summary

Cytogenetic studies of two cases of acute erythro leukaemia are reported In patient no 1 all metaphases of the marrow aspirates and the blood specimens cultured without phytohaemagglutinin were abnormal whereas lymphocytes from the peripheral blood cultured with phytohaemagglutinin had normal female karyotypes Three abnormal stem lines were found in the marrow aspirates of this patient The data strongly suggest that the erythrocytic and granulocytic precursors were derived from the same stem cell The chromosomal constitution of the second

patient was normal apart from structural abnormalities which were probably induced by 6 mercaptopurine

Acknowledgement

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From Department of Clinical Physiology (Head O Munck, M D) Glostrup Hospital Medical Department B (Head M Bjorneboe M D) and Department of Clinical Chemistry (Head P Lous M D) Bispebjerg Hospital Copenhagen Denmark

Diagnostic Significance of Hypogammaglobulinemia

By

Stig Bryde Andersen and Peter S Ward

Hypogammaglobulinemia is usually described as occurring in four forms 1 A primary congenital form which is hereditary and virtually confined to boys This is the classical agammaglobulinemia initially reported by Bruton (2) 2 Primary acquired hypogammaglobulinemia which occurs in adults of either sex with no recognizable hereditary factor 3 A secondary acquired form is encountered in patients suffering from malignant diseases of the reticulo endothelial system or from hypoproteinemia 4 A transient physiological form is present in infants 1 to 4 months old Numerous studies have been published on the occurence of hypogammaglobulinemia in various disorders No reports however have appeared concerning the diagnostic significance of this finding We have attempted to elucidate this problem

Methods

During a 6-year period a total of 9 469 paper electrophoretic analyses were carried out in Bispebjerg Hospital a general hospital in Copenhagen In 70 patients the serum gamma globulin was below 0 50 100 ml which represents the normal mean minus four times the standard deviation (table I)

Paper electrophoreses were carried out by the method of Laurell et al (8) With this method the beta peak is split into two components by the addition of calcium ions to the veronal buffer Protein fractions were determined spectrophotometrically by elution of paper-electrophoretic strips stained with bromophenol blue The gamma globulin concentration is underestimated by this technique because the stainability of gamma globulin is only 0 59 relative to that of albumin as unity (7)

Total serum protein concentrations were determined by the biuret method or by copper sulphate

Results

The patients were classified according to their clinical diagnoses (table II) None of them had primary hypogammaglobulinemia congenital or acquired and none had the transient physiological form Twenty patients (29 %) had malignant diseases of the reticulo-endothelial system Eight patients (11 %) had the nephrotic syndrome Twenty

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TABLE I Normal values for serum protein fractions in paper electrophoresis as determined by the method of Laurell et al (8)

	Laurell and Skoog (9)	Jarnum (5)
No of subjects	34	39
Total protein (g/100 ml)	7 1 ± 0 27	6 9 ± 0 37
Serum albumin (g/100 ml)	4 55 ± 0 18	4 45 ± 0 26
Serum γ globulin (g/100 ml)	0 87 ± 0 084	0 92 ± 0 108
Mean of serum γ globulin −4 times the S D	0 53	0 49

TABLE II Classification of 70 patients with hypogammaglobulinemia

	No	Frequency (%)
Malignant diseases of the reticuloendothelial system	20	29
Nephrotic syndrome	8	11
Disorders of the gastro-intestinal tract	26	37
Miscellaneous	16	23
Total	70	100

six patients (37 %) had disorders of the gastro intestinal tract and 16 patients (23 %) had miscellaneous disorders

The group with malignant diseases of the *reticulo endothelial system included* 7 patients with multiple myeloma 4 with Hodgkin s disease, 7 with chronic lymphatic leukemia 1 with Brill Symmer s disease and 1 with prostatic cancer and multiple bone metastases The 7 patients with multiple myeloma had paraproteinemia with beta mobility

The group with disorders of the gastro intestinal tract included 4 patients with protein losing enteropathy 2 with hypertrophic gastritis 4 with gastric cancer, 1 with colon cancer and 13 patients with benign gastric or duodenal disorders (5 had undergone partial gastrectomy) In addition 1 patient had ileus and 1 had melaena of unknown origin

Sixteen patients had miscellaneous disorders (table III) Four of these showed *uncompensated heart disease* 2 had attempted suicide and 2 patients suffered from anorexia nervosa The remaining 8 patients had various disorders apparently without common features

Discussion

Gamma globulin is synthesised in the reticulo endothelial system by plasma cytic cell lines (1 3 10) Accordingly the hypogammaglobulinemia in malignant disorders of the reticulo-endothelial system may be explained by a low synthetic rate

TABLE III Miscellaneous disorders with hypogammaglobulinemia

Sex	Age	Diagnosis	Total protein (g/100 ml)	Serum albumin (g/100 ml)	Serum glob (g/100 ml)
♀	65	Heart failure	6.4	4.43	0.34
♀	69	Heart failure	6.5	4.30	0.44
♂	77	Heart failure	5.6	4.05	0.47
♀	77	Heart failure	5.9	4.48	0.48
[illegible]	39	Suicide attempt	7.5	5.13	0.35
♀	51	Suicide attempt	6.1	3.26	0.50
♀	22	Anorexia nervosa	5.5	3.88	0.41
♀	21	Anorexia nervosa	6.1	4.47	0.42
♂	39	Alcoholism	6.5	4.20	0.44
[illegible]	62	Cholelithiasis	7.4	5.00	0.45
[illegible]	85	Liver cirrhosis	5.9	3.65	0.37
♀	34	Allergic reaction	5.7	3.67	0.35
♂	29	Methyl alcohol poisoning	6.0	3.94	0.43
♀	38	Crural lymphedema	5.2	3.61	0.43
♀	72	Crural fracture	6.07	4.08	0.47
♀	43	Fibromyoma	6.1	4.13	0.48

The low serum gamma globulin in the nephrotic syndrome is due mostly to loss of protein in the urine but probably also to an increased endogenous catabolic rate (4). In several gastro intestinal disorders hypogammaglobulinemia may be caused by a gastro intestinal loss of protein (6). But about half the patients of this study suffered from gastro intestinal diseases usually not associated with loss of protein.

The mechanism for the development of hypogammaglobulinemia cannot be explained in the group of patients with miscellaneous disorders. Total serum protein was low in several patients, suggesting that hemodilution might have caused the low serum gamma globulin. However this was not confirmed by determination of plasma volume.

It is essential to realise that this investigation has not attempted to show that the hypogammaglobulinemia in half the patients apparently is unexplained. The study is retrospective and the patients have not been examined so extensively as to warrant such a conclusion.

But our results emphasize the fact that about half of the patients with hypogammaglobulinemia are suffering from a disorder of a serious nature. Hypogammaglobulinemia therefore is a serious symptom, the nature of which should be cleared up — if possible.

Summary

During a six year period 9 469 electrophoretic analyses were carried out in a

general hospital in Copenhagen Hypo gammaglobulinemia was found in 70 patients Twenty nine per cent of these had malignant diseases of the reticulo endothelial system, 11 per cent had the nephrotic syndrome, 37 per cent had disorders of the gastro intestinal tract, and 23 per cent had miscellaneous disorders without common characteris tics *Hypogammaglobulinemia is a serious* symptom the cause of which should be cleared up

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From the University of Oslo, Institute of Hygiene, and the University of Bergen Institute of Hygiene and Social Medicine, Norway

Changes in Blood Pressure with Age

A Descriptive Analysis Based on a Cross sectional and a Longitudinal Study of Norwegian Men, 15—70 Years of Age

By

TOR BJERKEDAL and HAAKON NATVIG

Results of cross sectional studies of the relationship between blood pressure and age in adult population groups agree, in general, that the mean values of systolic and diastolic pressure increase with age (2, 3 4, 5, 8) The mean systolic pressure increases slightly after maturity up to the age of 40, and more rapidly thereafter The mean diastolic pressure, on the other hand, shows a slight but steady increase after maturity, up to the age of about 60 years In older age groups, a decrease in the mean value is often observed

Mean blood pressure values in different age groups are useful in giving a general idea of changes with age However, we would like to know whether blood pressure increases in every individual as he grows older, or whether the increase in mean values observed in cross sectional studies is caused by increasing pressures in only a small proportion of the population To answer such questions, longitudinal studies, preferably on unselected samples of the general population, would be needed Such studies are under way in various countries today

Reports on longitudinal studies of blood pressure in groups of persons who are not selected because of high blood pressure or related pathological conditions, are, however scarce at the present time Investigations of this kind (6, 11) have shown in general little change in mean values over the observation period, and about equal numbers of those examined have decreasing and increasing diastolic pressures Thus, there appears to be a discrepancy between findings from cross sectional and longitudinal studies The reasons for this discrepancy might be disclosed at least partly, by comparing in some detail results of the two types of studies

Such a comparison is attempted in the present report The data at hand are

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TABLE I Age distribution of 11 063 men included in a cross sectional study of blood pressure

Age in years	No of persons	Per cent
Less than 20		
(15—19)	724	6 5
20—29	2 340	21 2
30—39	2 948	26 6
40—49	2 252	20 4
50—59	1 819	16 4
60 and more		
(60—69)	980	8 9
Total	11 063	100 0

so called occasional blood pressure readings on male employees in various factories in Norway The analysis is purely descriptive, with no intention of providing suggestions as to what should be regarded as high blood pressure or hypertension, rather, it is aimed at estimating in quantitative terms in what proportion of the population blood pressures increase with age at what age, and how much

Material

The cross sectional material is derived from medical records collected in 1952 by The Board of the Industrial Health Service from physicians engaged in industrial health service programmes in 112 factories The records stem from results of periodic health control examinations offered the employees These records have been utilized in a number of investigations (1 7 9 12) and the referenced reports contain detailed information on the material

Included in the present study are records for 11 063 men 15—70 years of age Age distribution of the study population by 10 year age groups is given in table I The blood pressures were measured by the auscultatory method upon persons in sitting position with Hg manometer using a blood pressure cuff 12—13 cm wide The 5th phase (the complete cessation of the tone) was usually used for the diastolic measurement

The longitudinal material is derived from medical records for employees of three factories in Oslo Blood pressure readings most of which were made by one of the authors (H N), were obtained as in the cross sectional material during a routine health control examination and cover examinations performed from 1940 to 1956 Blood pressure was measured by the same method as in the cross sectional material

In 1956 medical records for current and past employees of the three factories were screened All records for male employees between 15 and 70 years of age when first examined were included in the material provided the interval between first and last recorded systolic blood pressure was at least 5 years None who fulfilled these requirements was excluded regardless of the reason for discontinuation of employment and consequent loss from observation

The material consists of records for a total of 459 men and table II gives information on age when first examined (by 5-year age groups) and the number of years (observation years) between first and last recorded systolic pressure Number of years of observation varies from 5 to 16 per individual is on the average 10 1 and totals 4 651 As shown in table III, the number of systolic readings totals 2 850 averages 6 2 per person and 0 51 per person year The latter figure means that systolic pressure was measured on the average about every other year after the first examination

Diastolic pressure was not recorded consistently in the early 1940 s and the number of such readings is therefore somewhat less than the number of systolic readings 2 759 compared with 2 850) Number of observation years for diastolic blood pressure is consequently slightly less

TABLE II Age when first examined and number of years between first and last recorded systolic blood pressure readings for 459 men in the longitudinal study

Age in years	No of persons	Number of observation years													Aver age
		5	6	7	8	9	10	11	12	13	14	15	16	Total	
15—19	48	6	7	6	10	5	2	6	4		2			401	8 4
20—24	50	8	4	5	5	3	5	3	8	1	1	7		477	9 5
25—29	71	6	9	6	5	5	7	10	6	2	7	5	3	710	10 0
30—34	72	6	8	4	4	5	5	7	6	5	14	8		763	10 6
35—39	60	2	3	2	3	6	4	5	13	3	8	9	2	689	11.5
40—44	52	3	4	3	5	3	1	6	8	2	13	4		567	10 9
45—49	37	5	2		4	4	1	6	2	1	7	3	2	393	10 6
50—54	37	3	5	4		2	4	7	2		7	3		375	10 1
55—59	14	2	3			2	2	1	3		1			127	9 1
60—64	15	4	1	2		1	4	1		1	1			127	8 5
65—69	3	1		1			1							22	7 3
Total	459	46	46	33	36	36	36	52	52	15	61	39	7	4 651	10 1

Outline of analysis

Cross sectional material Analysis of the cross sectional material is limited to calculations of mean systolic and diastolic pressure, and of standard deviations of the distributions of blood pressures in the various age groups These values are compared with corresponding statistics reported by others from similar studies

Also presented are cumulative frequency distributions of systolic and diastolic values for various age groups they are cumulated from the highest to the lower value and are plotted on probit paper In the graph, they will appear as straight lines provided blood pressures are distributed approximately normally

Longitudinal material It will be apparent from the brief description given that the material is not suitable for an elaborate analysis Men enter the study at different ages and in different calendar years they are observed for a varying number of years and blood pressure is measured an unequal number of times with varying intervals between readings Analysis is, for this reason, approached in the most simple way

First mean systolic and diastolic pressures at the first and last examination were obtained for each of the 5 year age groups These mean values are compared with those found in the cross-sectional material To account for some of the observed differences between the two materials particularly with respect to diastolic pressure distributions of diastolic values in selected age groups of the two materials are compared Moreover a comparison is made of average differences between the first recorded diastolic pressure and

TABLE III Number of recorded systolic blood pressures by number of observation years for 459 men

No of observation years	No of persons	Number of recorded systolic blood pressures						
		2	3	4	5	6	7	8
5	46		7	25	8	6		
6	46	2	3	14	15	7	5	
7	33	1	4	4	15	2	4	3
8	36		4	8	13	3	1	5
9	36		1	4	16	5	4	4
10	36			3	17	9	4	1
11	52		1	3	20	11	9	3
12	52			1	14	11	22	
13	15				5	4	1	
14	61			1	9	13	18	6
15	39				2	5	8	9
16	7					1		3
Total	459	3	20	63	134	77	76	34

subsequent readings recorded 1 year 2 years, etc, up to 16 years after the first. Age when first examined is disregarded in this comparison because results of the cross sectional study indicated that the increase in diastolic pressure was about the same from one age group to the next throughout the main part of adult life However, all persons were not observed for an equal number of years, and not examined every year Thus, the comparisons of the average differences are based on different subgroups of the study population For the first 5 years, the subgroups are probably representative of the total study population because all 459 men included in the study were observed for at least that time and probably had an even chance of being examined any particular year during that period From the 6th observation year onwards however, losses from observation due to discontinuation of employment will most probably cause subgroups to be non representative

The next step in the analysis deals with differences between first and last recorded readings, and as data in table II show, the interval between the readings averages about 10 years Analysis is based on the distribution of such differences and on the correlation between the differences and the value of the first recorded pressure For diastolic pressure, distributions as well as correlations were very much alike for the various 5-year age groups, and are presented for all age groups combined On the other hand, the distributions of differences between first and last recorded systolic pressure changed markedly with age They provided a basis for estimating the proportion of persons in the various age groups in whom the systolic blood pressure probably increased during

included in the longitudinal study

9	10	11	12	13	14	15	Total	Average per person	Average per person/year
							197	4 3	0 66
							221	4 8	0 63
							169	5 1	0 59
2							192	5 3	0 54
2							207	5 8	0 53
	1	1					208	5 8	0 48
2		3					319	6 1	0 47
1	1	2					335	6 4	0 45
1	1	2	1				109	7 3	0 48
	1	2	4	3	4		476	7 8	0 49
4			2		7	2	356	9 1	0 54
1	1		1				61	8 7	0 48
13	5	10	8	3	11	2	2 850	6 2	0 51

the period of observation, and for obtaining rough estimates of the magnitude of the increases

The last step in the analysis is an attempt to investigate whether persons who show an increase in systolic blood pressure during a 10 year observation period would have shown a further increase had they been observed for another 10 years

Results

Cross sectional material Mean systolic and diastolic blood pressure values for different age groups in the present material are compared in fig 1 with mean values reported by others from similar studies It will be apparent that mean systolic and diastolic pressures increase with age in much the same way whether measured on population groups in Bergen (2) or Muscogee County (3), on patients referred to outpatient clinics of St Mary s Hospital in London (4), or on employees in American (8) or Norwegian factories Mean systolic pressure can be seen to increase little between the ages of 20 and 40 but thereafter shows a progressive increase The mean diastolic pressure apparently increases steadily, about the same amount from age group to age group However, for the oldest men mean value shows a tendency to decrease

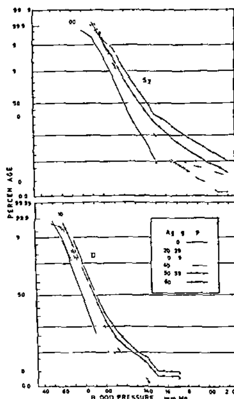
The mean values for the same age groups in the different investigations differ somewhat — a point well illustrated by the curves in fig 1 representing mean systolic pressures found in the two investigations conducted in Bergen These two curves are almost parallel, which means that systolic readings were somewhat lower and equally lower in all age groups in investigation "2 than in investigation "1 Even

TABLE III Number of recorded systolic blood pressures by number of observation years for 459 men

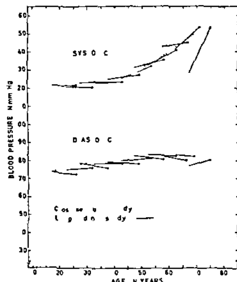
No of observation years	No of persons	Number of recorded systolic blood pressures						
		2	3	4	5	6	7	8
5	46		7	25	8	6		
6	46	2	3	14	15	7	5	
7	33	1	4	4	15	2	4	3
8	36		4	8	13	3	1	5
9	36		1	4	16	5	4	4
10	36			3	17	9	4	1
11	52		1	3	20	11	9	3
12	52			1	14	11	22	
13	15				5	4	1	
14	61			1	9	13	18	6
15	39				2	5	8	9
16	7					1		3
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F g 3 Cumulat ve frequency d str but ons of sys ol c and d astol c blood pressure read ngs for men by age



F g 4 Compar son of the relat onsh p found between age and mean systol c and d as ol c blood pressure read ngs in a cross-sect onal and long tud nal study of men.

graph it can be seen that systolic pressures in the youngest age group 15—19 years are approximately normally distributed so that the curve representing this distribution is almost a straight line The changes in the distribution that occur with age may be described as being partly a shift toward h gher blood pressure values partly a building up of an excess frequency of medium high and high values Distributions for diastolic values shown in the lower section of fig 3 change with age as described for systolic values The build ing up of excess medium high and high values is much less however

It seems appropriate at this point to speculate briefly on how these changes may have come about Although a cohort effect cannot be disregarded entirely (5) we may reasonably assume that at least the major part of the observ ed changes reflect those that occur in individuals as they grow older On the basis of this assumption changes may be accounted for in two ways Either blood pressure (systolic as well as diastolic) increases with age in all individuals and proportional to the value of the pressure at the outset of the period or blood pressure increases with age in only a fraction of the population and may occur in time regardless of the initial value Obviously one can hope to disclose what actually takes place only by observing the same individuals over a prolonged period

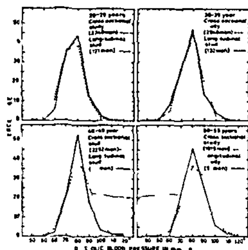


Fig 5 Distributions of diastolic blood pressure readings in a cross sectional study and the distributions of the first diastolic blood pressure reading in a longitudinal study for four 10 year age groups of men

Longitudinal material Comparison with the cross sectional material The relationships between age and mean systolic and diastolic pressure found in the two materials are compared in fig 4 The cross sectional material is represented by the broken lines, the longitudinal, by short solid lines, one line for each 5 year age group, drawn by connecting the mean value of the first and the last recorded blood pressures for all persons in the group, taking into account both the average age when first examined, and the average number of observation years

The two materials agree fairly well with respect to the relationship between mean systolic pressure and age, the short solid lines follow the broken line reasonably closely However, the first four solid lines are almost horizontal, indicating no increase in mean systolic pressure during the observation period for persons less than 35 years of age when first examined, while a slight increase is observed in the cross sectional material The line at the extreme right in the diagram, representing the oldest age group, is based on too few observations to be reliable

The two materials disagree to some extent with respect to the relation between mean diastolic pressure and age First of all, it can be seen in fig 4 that mean values at the first examination of men older than 30 years are always lower than for those of corresponding ages in the cross sectional material, and that the difference between the two materials in this respect tends to increase with age It may be noted also that most of the short solid lines have a downward slope, indicating a decrease rather than an increase in mean diastolic pressure over the observation periods of about 10 years

A comparison of distributions of diastolic values in selected age groups of the two materials shown in fig 5 gives a clue to why mean values are lower in the longitudinal material for men older than 30 years when first examined While the distributions for men 20—29 years of age are very much alike, a slight "deficit" of readings of about 90 mm Hg can be seen for the age group 30—39 in the longitudinal material, and for the two older age groups there is a more marked "deficit" of medium high and high values

These "deficits", which could account for lower mean values in the longitudinal material, are probably a result of selection Height of diastolic pressure apparently influenced whether or not a

person was included in the longitudinal material — to a larger extent than might be the case in the cross sectional material It is possible that persons over 40 years of age with medium high or high diastolic pressure had not applied for work in the three factories from which the longitudinal material was obtained It is also possible, in fact more likely, that men with the higher pressures had been employed but had not worked the minimum of 5 years required for inclusion in the material

The reason no increase in mean diastolic pressure was found in the longitudinal material appears from a comparison of the curves shown in fig 6 to be partly a systematic difference between first and subsequent readings partly a selective loss from observation of persons in whom diastolic pressure increased One curve represents average differences between the first diastolic reading and subsequent readings recorded 1 year, 2 years etc up to 16 years after the first examination The two other curves represent a constant yearly increase of 0 3 mm Hg as found in the cross sectional material

That the first reading must have been somewhat higher than subsequent readings is indicated by the fact that average differences are negative during the first 5 years That the absolute value of the negative difference is reduced from the second to the fifth year is taken to indicate that the systematic difference between first and subsequent readings is counteracted by a real increase in mean diastolic pressure This increase, as can be seen corresponds to that observed in the longitudinal material

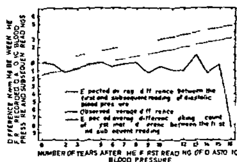


Fig 6 Average differences between the first recorded diastolic blood pressure reading and subsequent reading for 459 men observed from 5 to 16 years.

The selective loss from observation of persons in whom diastolic pressure increased is indicated by the fact that average differences between first reading and readings after the fifth year of observation fail to show the expected trend Such a selective loss is not necessarily great in terms of actual number of persons or in terms of a percentage of the study population It may be calculated that if 1 per cent of the study group is lost per year because of increasing diastolic pressure the total increase in the 1 per cent would not need to be more than 30 mm Hg to counteract a yearly increase in the mean value of 0 3 mm Hg Although such a calculation is quite hypothetical it would nevertheless suggest that it might not be unrealistic to suggest that diastolic blood pressure apparently does not change with age in the majority of adult males

Differences between first and last recorded diastolic blood pressure No change in diastolic pressure with age in the majority of adult males does not mean of

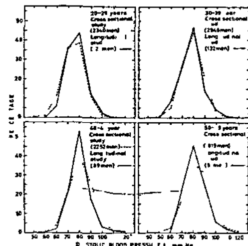


Fig 5 Distributions of diastolic blood pressure readings in a cross sectional study and the distributions of the first diastolic blood pressure reading in a longitudinal study, for four 10 year age groups of men.

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may note as exceptional (in addition to the three men with increases of 40 mm Hg) the record showing a reading of 120 mm Hg at the first and 130 mm Hg at the last examination Limiting our concern to increases only, we may on intuitive grounds suspect 1 to 2 per cent of the differences to indicate real increases in diastolic blood pressure over periods which averaged about 10 years Of course, the 1—2 per cent give no more than a very rough, in fact a minimum, estimate of what we might expect to find in the way of increases in diastolic blood pressure in the general population of adult males over 10 year periods

TABLE IV Correlation between the first recorded diastolic blood pressure reading and the difference between first and last recorded readings in 459 men observed on the average for about 10 years

F RST RECORDED D ASTOLIC BLOOD PRESSURE (mm Hg)

D FFERENCE IN mm Hg BETWEEN FIRST AND LAST RECORDED D ASTOL C BLOOD PRESSURE (LAST READ NG THE F RST)	50	60	70	80	90	100	110	120	130	TOTAL
45										
+40			1	2						3
35										
30			1							1
25		2								2
20		7	2	5	1					5
15		3	5	2						0
10		10	38	20	7	2		1		78
5		2	8	9	2	1	1			23
0		4	55	74	18	1	1			153
5			8	18	6	1				33
10			15	65	24	6				110
15			1	6	1					8
20				10	5	2	1	1		19
25				1			1			2
30				1		1				2
35										
40										
45										
TOTAL		28	134	2 3	64	16	4	2		459

Differences between first and last recorded systolic pressure With few exceptions, differences between first and last recorded systolic readings for men not observed beyond the age of 40 years reflect random variations in measurements Apparently systolic pressure in the majority of adult males does not change appreciably up to the age of 40 This interpretation is the basis for the following analysis for persons observed beyond 40

It is assumed that random variations in measurements of systolic pressure in groups of persons in which no real increase in pressure occurs are independent of age (as in fact was found to be the case for diastolic pressure) Thus, by comparing distributions of differences between first and last measurements for the older age groups with the distribution for the group of men not observed beyond the age of 40, we may obtain estimates of the proportion in whom differences between first and last recorded systolic reading is likely to reflect a real change (increase) in pressure

The distribution for the group of men not observed beyond the age of 40 can be seen to be almost symmetrical from fig 8 The highest frequency is at zero difference and the range from minus 45 to plus 45 mm Hg with only one extreme value of plus 60 For those observed beyond the age of 40 the distributions clearly show an increasing frequency of positive differences with increase in age at first examination Estimates of the excess frequencies of positive differences are given in table V The table also includes estimate of the average increase, and results of calculations intended to show to what extent these increases would influence mean systolic pressure for the total group over a period of 10 years

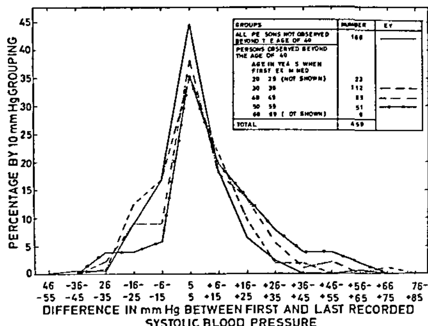


Fig 8 Distribution of differences between first and last recorded systolic blood pressure readings for selected groups of a total of 459 men observed on the average for about 10 years

TABLE V Estimates of the percentage of men that will show an increase in the systolic blood pressure during an observation period of on the average about 10 years

Age in years when first examined	No of persons	Estimated percentage of men that will show an increase in systolic blood pressure	Average size of increases (mm Hg)	Influence on average systolic blood pressure over a 10 year period (mm Hg)	Difference in average systolic blood pressure found in the cross-sectional study between two successive 10-year age groups
30—39	112	17 2	15 7	2 7	2 8
40—49	89	28 8	21 2	6 1	6 2
50—59	51	42 2	24 8	10 5	10 8

For men who are in their thirties when first examined and who are observed beyond the age of 40, about 17 per cent are estimated to show an increase in systolic pressure during a 10 year interval The average increase in systolic pressure in this 17 per cent is estimated to be *15 7 mm Hg, which will cause*

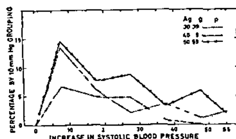


Fig 9 Distributions of estimated increases in systolic blood pressure for three age groups of men observed on the average for about 10 years

the mean for the total group to increase 2 7 mm Hg over the 10 year period Similar estimates for men 40—49 and 50—59 years of age when first examined indicate that the percentage in whom an increase will be observed during 10 year periods will increase with age The average increase will become greater, as will the influence on mean systolic pressure for the total group over the 10 year period Differences between mean systolic pressures observed in the cross sectional study are added to table V (to serve as a check on these estimates) The agreement between observed and estimated change in mean systolic values is very good and suggests that the estimates might not be unrealistic

This part of the analysis is summarized in *fig 9* by the distributions of increases in systolic pressure estimated to have occurred over a 10 year period in the different age groups The distributions illustrate fairly well not only that the percentage of men in whom an increase in systolic pressure will occur over a 10-year period becomes greater with age, but also that an increasing

TABLE VI Correlations between the first recorded systolic blood pressure and the difference between first and last recorded readings in 166 men not observed beyond the age of 40 years (upper section) and in 140 men 40—59 years of age when first examined and observed on the average about 10 years

FIRST RECORDED SYSTOLIC BLOOD PRESSURE (mm Hg)

166 me not observed beyond the ge f 40 yea s

SYSTOLIC BLOOD PRESSURE (LAST READING THE FIRST)	80	90	100	110	120	130	140	150	160	170	180	190	200	TOTAL
75														
70														
65														
60					1									1
55														
50														
45														
40														
35				1	1									2
30			1	1										2
25			1											1
20			3	4		2		1						10
15			1	5		1								7
10			3	10	7	2	1	1						24
5		1	1	1	3		1							7
0			1	16	21	12	3	3						56
5				3	5	3								11
10				1	14	8	3							26
15					1			1						2
20					2	7	2							11
25						1	3							4
30														
35								1						1
40						1								1
45														
50														
TOTAL		1	11	42	55	37	13	7						166

140 men g 40 59 ye wh 1 1 min d

DIFFERENCE IN mm Hg BETWEEN FIRST AND LAST RECORDED	80	90	100	110	1 0	130	140	150	160	170	180	190	200	TOT L
TOTAL	1	1	3	14	41	36	35	10	2	2	2	2	1	140
75														
70				1										1
65						1								1
60														
55								1						1
50			1		1		1							3
45						1								
40	1					1								2
35					1	2	1							4
30			1		1	1		2						5
25					1		2							3
20		1			8	5	2							16
15				1		1	1							3
10			1	4	8	7	2	1						23
5				1	7		5	1						14
0				4	7	6	5	2	1	1	2		1	29
5					3	5	1							9
10				1	3		4	1		1				10
15						1								1
20				1	1	3	1	1	1			1		9
25						1								1
30				1		1		1				1		4
35														
40														
45														
50														

FIRST RE ORDED SYSTOLIC BLOOD PRESSURE (mm Hg)

percentage will show considerable changes of plus 45 mm Hg and more

An attempt was made to investigate whether persons who show an increase in systolic blood pressure during a 10 year period would have shown a further increase had they been observed for another 10 years With respect to this

question, the longitudinal material is in fact no more than cross sectional Nevertheless, an indication as to what might occur is obtained by comparing correlations between the first recorded systolic pressure and the difference between the first and the last recorded reading in two groups of men those not observed beyond the age of 40 years, and those who were 40 years and older at the first examination

The correlations are quite different in the two groups, as can be seen from table VI The older group includes a number of men with medium high and high systolic values that were not found in the younger group furthermore, the inverse correlation so clearly seen in the younger is obscured These differences, it seems, indicate that in the group of 40—59 year olds, there are persons in whom blood pressure must have increased since they were in their thirties, and that it is particularly among those persons that differences between the first and last recorded pressure indicate that a real increase has occurred during the observation period If this interpretation is accepted, it would mean that systolic blood pressure increases in only slightly more than 50 per cent of adult men up to the age of about 65 years

Discussion

It is realized that there are shortcomings in the materials used for the present study One of these is that the study group is not a representative sample of the adult male population The materials were simply extracted from medical records for men employed in factories and firms with industrial health service programmes The influence on blood pressure of the selection that goes into choice of employment and the effect of occupation cannot be assessed A much more serious shortcoming, however, was created by selecting for the longitudinal material only those men who had blood pressure records that extended for periods of 5 or more years The effect of that procedure, we now realize, tends to eliminate those in whom blood pressure increases with time

To be considered also is the fact that blood pressures on which the study is based were measured with no plan that they should later be used for research purposes Some changes in the procedures of routine health examinations would be needed in order to obtain the standards of measurements and procedures required for longitudinal investigations It would seem, for example, that to engage trained technical personnel for making the observations should make it possible to standardize the whole data collecting process including the important requirement of insuring that no record of previous results is available to the examiner at the time he is making the observation If such changes were implemented, observations made during routine health examinations would, no doubt, provide an extremely valuable source of information for large scale longitudinal studies, in addition to their use for clinical and preventive services In terms of efforts and cost, utilization of these sources would be highly advantageous compared with

specially designed long term prospective investigations

The analysis of the longitudinal material for the present paper employed only the most conventional statistical methods which meant that a large amount of data was left unused To utilize all data quite advanced and elaborate statistical techniques would have had to be employed which, in view of the shortcomings of the material would seem not to have been desirable Nevertheless, a primary aim of such a longitudinal investigation should be to define criteria by which individuals whose blood pressure shows a tendency to increase can be separated from those with 'stable" pressures With such criteria at hand, factors mainly responsible for an increase in pressure could most effectively be investigated, and the clinical and prognostic significance of an increase could be evaluated To consider magnitude and rate of change in blood pressure with time in addition to the actual level of the pressure would no doubt add a fruitful dimension to the study of cardiovascular renal diseases

Summary

An analysis is presented of blood pressure measurements on men 15—70 years of age employed in Norwegian factories and firms with industrial health services It is based on two materials one included 11 063 men for whom information on a single blood pressure determination was available the other 459 men on whom blood pressure readings had been repeated over a period of at least 5 years and on the average slightly more than 10 years

A main part of the paper deals with a comparison of the cross sectional and longitudinal materials Differences were observed, particularly with respect to diastolic pressure, which suggest that the first reading in a series tends to be higher than subsequent readings, and that men who show increases in diastolic pressure tend to be lost from observation earlier than those whose pressure remains essentially unchanged

Results of the analysis also suggest that the increase with age in mean diastolic pressure found in the cross-sectional material is caused by a small proportion of the group in whom pressure increases Diastolic pressure apparently does not change appreciably in the majority of men throughout adult life The increase with age in mean systolic pressure found in the cross sectional material up to the age of 40 years may be accounted for in a similar way After the age of 40 however, the proportion of men in whom systolic pressure increases will rapidly become greater It seems, nevertheless, that systolic pressure may not change appreciably in about 50 per cent of men up to the age of about 65 years

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From the Department of Medicine (Head C L H Majoor, M D) St Radboud Hospital University of Nijmegen Nijmegen The Netherlands

Increased Serum Alkaline Phosphatase Activity in Pulmonary Infarction

By

J H Dijkman and P W C Kloppenborg

During one of the clinico pathological conferences at the Massachusetts General Hospital in 1963 (1) the case of a female patient was discussed in which an increased serum alkaline phosphatase activity had offered diagnostic difficulties The tentative clinical diagnosis of primary carcinoma of the liver was finally made Multiple pulmonary infarctions were found at postmortem examination No correlation was made between the increased alkaline phosphatase activity and these pulmonary infarctions No satisfactory explanation was offered for the increased alkaline phosphatase activity

In a more recent conference at the same hospital (1965) (8) a new case of multiple pulmonary infarctions with high serum alkaline phosphatase values was discussed Again no explanation was presented for this high serum enzyme level

In the past few years we have found increased serum alkaline phosphatase activity in several patients with pulmonary infarction These findings confirmed the report of the Finnish investigator Nikkilä (11), who was the first to point out the presence of increased alkaline phosphatase activity in patients with pulmonary embolism

Since in our opinion Nikkila s report did not receive the attention it deserved it seemed useful to publish our findings

Material and methods

The patients

The serum alkaline phosphatase activity was measured in 26 out of 34 patients admitted during 1960 and subsequent years and considered to be suffering from pulmonary embolism with radiologically demonstrable pulmonary changes (pulmonary infarction) In 6 of these 26 patients the activity was measured only once In the remaining 20 cases determinations were made serially in the first patients because a pathologically increased value was found acciden

Submitted for publication December 30 1965

The results of this investigation were presented at the meeting of the Dutch Society for the study of diseases of the lungs and tuberculosis held in Nijmegen on May 23 1964

TABLE I Clinical and roentgenological data

Patient no	Age	Sex	Observation period	Previously immobilised	Sudden chest pain	Haemoptysis	Manifest thrombosis	ECG-changes
1	68	♂	27–7–60 to 1–9–60	Ablatio retinae Operation 20 7–60	26–7–60	+	Right leg	–
2	70	♂	6–12–60 to 24–3–61	—	4–12 60	+	—	–
3	52	♂	26 12 61 to 23–2–62	—	25–12–61	–	Left leg	+
4	54	♂	10–1–62 to 23–2–62	—	Not clear	+	Right leg	–
5	80	♂	23–2–62 to 5–5–62	—	Not clear	+	–	–
	81	♂	30 8–63 to 1–10–63	—	30–8–63	+	–	+
6	49	♀	31–7–62 to 28–8–62	Intercostal neuralgia	Not clear	–	–	–
7	25	♂	30–5–63 to 10–7–63	Hernia diaphragmatica Operation 9–5 63	25–5 63	+	–	+
8	32	♀	18–10–63 to 5–12 63	Cholecystectomy 2–10–63	15–10–63	–	-	+
9	59	♂	11–11–63 to 7–12–63	Acute myocardial infarction on 11–11–63	17 11–63	+		+
10	72	♂	2 12–63 to 9 1–64	—	1–12–63	+	Left leg	–
11	58	♀	5–1 64 to 12–2–64	Varicose ulcer	4–1–64	+	Left leg	–
12	69	♀	5–3–64 to 6–5–64	Rheumatoid arthritis	1–3–64	–	-	–
13	85	♂	26–3–64 to 6–6–64	Urine retention Cystopyelitis	20–4 64	+	–	–
14	74	♂	27 5–64 to 20–6–64	Arthrodesis right hip	Not clear	+	Right leg	–

Central venous pressure (cm H_2O)		Roentgenological signs		Duration of fever (days)	Treatment	Other diagnoses
On admission	Later	Pulmonary infiltration	Gall bladder			
R-3½	R-5	Left lower field pleural effusion	N[1]	26	Ac[3] Ab[4] up to 8-8 Salt restriction	Hypertrophy of prostate Urine retention Transient impairment of renal function
R+2	R-6½	Both lower fields pleural effusion	N[2]	70	Ac since 15-2 Ab from 5 to 17-3 Salt restriction Digitalis	Atrial fibrillation Pulmonary congestion
R 1	R-5	Both lower fields, pleural effusion	N	24	Ac Salt restriction	S.L.E.
R-4	R-4	Both lower fields pleural effusion	N	11	Ac Salt restriction Digitalis Tolbutamide	Atrial fibrillation Diabetes mellitus
R 6		Left upper lung field	N	25	Ab from 2 to 13-3	Gout
R 2	R-6	Left lower field pleural effusion	N	4	Ac Ab up to 11-9 Digitalis	Gout Transient atrial fibrillation
R 6		Right lower field pleural effusion	N[1]	10	Ac since 14-8 Ab up to 10-8	Gibbus resulting from tuberculous spondylitis
R-5		Right lower field pleural effusion	N	14	Ac Ab up to 17-6	—
R 6		Right lower field	N chol angiogram	17	Ac Ab up to 29-10	—
R 5	R 7	Right lower field	N	3	Ac Ab from 18 to 30-10 Salt restriction	Myocardial infarction
R-6		Right lower field	N	3	Ac Ab up to 14-12	Carcinoma of prostate (not metastasising)
R	R-4½	Both lower fields	N	12	Ac Salt restriction Digitalis Diuretics	Mitral stenosis and insufficiency Atrial fibrillation
R-4 /		Right lower field	N	4	Ac Hydrochloroquin	Rheumatoid arthritis
R-4	R-5	Right lower field pleural effusion	N	6	Ac Ab up to 1-5	Transient atrial fibrillation Impairment of renal function Anaemia
R 5	R-6	Left lower field	N	2	Ac	Coronary insufficiency Coxarthrosis

tally In later cases the serum alkaline phos phatase activity was followed deliberately to get information about the pattern of rise and fall of enzyme values that may occur

Laboratory methods

The serum alkaline phosphatase activity was determined using Bessey s original method (2) Normal values in our laboratory were calculated from the results of determinations performed in the serum of adult out patients of all age groups and both sexes In 85 successive patients without disease of bones joints liver gallbladder or lungs and without malignant disease or thrombo embolic mani festations values were found ranging from 1 0 to 3 2 mmol U with a mean of 1 9 mmol U In a larger group of determinations made in the sera of 1 126 out patients during two successive years regardless of the kind of disease 64 % of the values were found be tween 1 2 and 2 8 mmol U around a mean of 2 0 mmol U 4 % gave results between 0 4 and 1 2 mmol U Accepting a symmetri cal distribution of normal values (standard deviation 0 5 mmol U) a highest normal value was calculated at 3 6 mmol U (p 0 001) In this paper values over 4 0 mmol U were considered to be pathological Bilirubin (10) serum transaminase (3 15) thymol turbidity (9) and serum protein (5) determinations were carried out by routine procedures with minor modifications The ESR was determined according to Wester gren Plasma bilirubin values exceeding 0 8 mg/100 ml (total) and serum transaminase activities exceeding 40 U were considered abnormal

Results

In 6 patients, in whom the serum alka- line phosphase activity was measured only once, a normal value was found 1, 3, 8, 9, 30 and 35 days after acute pul monary embolism

Pathologically increased values of the alkaline phosphatase activity were found in 18 of 20 patients, in whom the deter mination was carried out several times In 4 cases it seemed possible that the increased activity was correlated with affections of the liver or gallbladder These cases are left undiscussed

Tables I and II present the principal data of history, clinical and radiological examination, treatment, clinical course and laboratory findings in the remaining 14 patients Patient No 5 was hospi talized twice

In 11 cases the diagnosis of pulmonary embolism was beyond doubt In 8 of these cases, breathing became acutely painful shortly after operation (patients Nos 1, 7 and 8), or after immobilization for other reasons (patients Nos 9, 11, 12, 13, 14) Three patients (Nos 3 4 and 10) developed pulmonary changes in association with thrombosis of the leg In 3 other cases (Nos 2, 5 and 6) the possibility of pulmonary embolism was not considered initially The further

Table I Cont (notes)

[1] Measurements as described by Borst J G G & Molhuysen A Exact determination of the cen tral venous pressure by a simple clinical method Lancet *2* 304 1952 About 90 % of normal values in males and females are found between R-4 5 and R-8 5 cm

[2] Not investigated

[3] Anticoagulants

[4] Antibiotics

[5] Normal contrast no stones

TABLE II Biochemical data

	Serum alkaline phosphatase			Liver function tests			
Patient no	On admission	1 to 3 wks later	At discharge	Bilirubin	SGOT/SGPT	Thymol turbidity	Serum electrophoresis
1	3 5	6 8	3 5	N[1]	N I [2]	2 2	N
2	N I	6 5	2 7	N	25/15	1 3	N
3	N I	12 2	3 2	N	40/62	2 1	N
4	N I	6 2	3 5	N	40/33	0 3	N
5	10 9	8 7	2 5	N	N I	2 2	N
	3 1	15 0	5 1	N	42/36	1 9	N
6	N I	7 1	2 5	N	19/24	0 3	N
7	2 3	8 8	2 7	N	114/125	0 5	N
8	10 0	8 7	4 0	N	96/88	2 5	N I
9	N I	7 3	3 9	N	41/26	2 4	N I
10	4 0	5 8	4 2	N	29/5	1 4	N
11	3 5	5 2	2 7	N	33/13	0 8	N
12	2 9	5 1	2 4	N	80/65	4 4	N
13	3 8	6 3	3 4	N	105/39	2 4	Transient hypoalbuminaemia
14	9 3	5 9	4 0	N	25/20	1 1	N I

[1] Plasma bilirubin not elevated
Not investigated

course indicated however that pulmonary embolism had been the cause of the symptoms

Haemoptysis occurred in 10 of 14 patients A pleural friction rub was heard in five Radiologically pulmonary changes were demonstrated in all cases Six of the 14 patients developed distinct symptoms of femoral vein thrombosis None showed signs of shock The ECG showed transient evidence of myocardial ischaemia in 5 cases Patient No 9, in addition showed dextrorotation of the cardiac axis None of the patients showed signs of acute cor pulmonale The other clinical diagnoses in these cases are listed in the final column of table I No patient died

Eleven patients were immediately treated with the anticoagulant acenocumarol (Sintrom) In cases 2 5 and 6 however, this medication was not started until later Patients Nos 1 2 5 6 7, 8 9, 10 and 13 were given antibiotics, because the clinical condition seemed to require this This treatment had no appreciable effect on the symptoms or radiological changes

In all 14 patients the increase in serum alkaline phosphatase activity was transient (table II) and fell to within or just above normal limits after treatment In

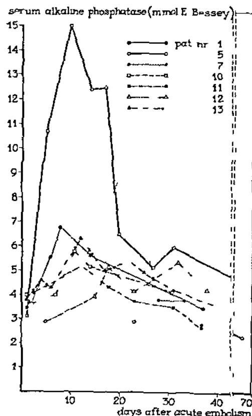


Fig 1 Rise and fall of serum alkaline phosphatase in 7 patients after acute pulmonary embolism with subsequent infarction

7 cases (Nos 1, 5 7, 10, 11 12 and 13) in whom the date of onset of the pulmonary embolism could be established on clinical grounds, a rise and fall in the enzyme activity was recorded The values found in this group are shown plotted against time in fig 1 Both the height of the maximum and the time at which this maximum was attained prove to have been subject to considerable individual variation

The serum bilirubin concentration was normal in all patients (table II) In 11 cases cholecystography was carried out to eliminate affections of the biliary tract as a cause of the increased alkaline phosphatase activity (table I) A normal gallbladder picture, without filling defects caused by stones, was obtained in all patients examined

Case report

Patient No 5

An 80 year old man was admitted on February 16th 1962, with an opacity in the upper lobe of the left lung suggestive of bronchial carcinoma No signs of thrombosis were found The body temperature was 37 6° C

The LSR was greatly increased (103 mm) The leucocyte count and differential count were normal The alkaline phosphatase activity was found to be unmistakably increased (10 9 mmol U) The plasma bilirubin and the thymol turbidity of the serum were normal Pyrexia occurred a few days after admission The patient expectorated purulent sputum which later became blood tinged Bronchographic and bronchoscopic examination yielded no findings supporting the diagnosis of bronchial carcinoma The pulmonary anomaly was regarded as the result of an inflammatory process and antibiotic medication was consequently started The pulmonary infiltration gradually disappeared The ESR and the alkaline phosphatase activity returned to normal values

After his discharge from the hospital on May 5th 1962 the patient remained free of symptoms until August 30th 1963 Om that day pain in the left flank commenced abruptly Retrosternal pain radiating to the left shoulder started a few hours later

At admission the pulse was regular and there were no signs of shock The ECG indicated coronary insufficiency without infarction Physical and radiological examination disclosed an infiltration in the left lower lobe Signs of thrombosis were absent

During the first few days after admission the temperature rose (fig 2) and breathing caused violent intermittent pain in the left flank where a pleural friction rub was heard

The central venous pressure was increased but no oedema was found Transient atrial fibrillation was observed Anticoagulant therapy was instituted on September 2nd 1963 after a few days of penicillin medication Pain and dyspnoea diminished and the venous pressure returned to normal as did the temperature The pulmonary infiltration gradually disappeared Fig 2 shows that the ESR and the alkaline phosphatase activity reached a maximum on the 10th day after admission and subsequently diminished

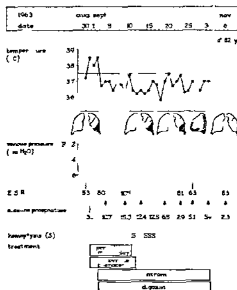


Fig 2 Some clinical radiological and laboratory data in a patient with acute pulmonary embolism (patient No 5 in table I)

Discussion

The combination of increased alkaline phosphatase activity and a normal serum bilirubin concentration is a common feature of a number of hepatic processes (primary carcinoma metastatic carcinoma, abscess of the liver) and osteopathies None of our patients showed signs suggestive of these conditions

Congestion of the liver with increased alkaline phosphatase activity and a normal serum bilirubin level is unusual In 34 of 50 patients with a congested liver resulting from cardiac decompensation Sherlock (12 13) found serum bilirubin levels above 1 mg/100 ml The serum alkaline phosphatase activity however was normal in 45 of these 50 patients

None of our patients showed signs of hepatic congestion at the time of maximal serum alkaline phosphatase activity In 4 cases (Nos 2, 3 5 and 11, cf table I) an increased venous pressure was found on admission In 2 of these a normal alkaline phosphatase activity was established on the same day The venous pressure in these 4 cases was normalized within a few days At the time of the maximum alkaline phosphatase activity none of the patients showed venous hypertension

In view of the above it is contended, that the combination of increased alkaline phosphatase activity and a normal serum bilirubin concentration in the presence of pulmonary changes should bring to mind the possibility of pulmonary infarction Our preliminary impression is that increased alkaline phosphatase activity following pulmonary infarction is the rule rather than an exception In only two of 20 carefully followed up patients was this phenomenon not seen In one of these cases the alkaline phosphatase activity did increase but not to pathological values (from 1 7 to 3 1 mmol U)

The question may be raised if the observed phenomenon does occur in other types of acute pulmonary disease Although we have

not yet investigated this point thoroughly we have determined repeatedly the serum alkaline phosphatase activity in 5 patients with lobar pneumonia In 3 of them a slight increase (from 1 9 to 3 5 2 6 to 3 9 and 3 2 to 3 9 mmol U) was found with maximal values in the second week of the illness, in the sera of the two other patients no rise was apparent

It is not possible to explain with any degree of certainty the increase in serum alkaline phosphatase activity in patients with pulmonary infarction When a branch of the pulmonary artery is occluded a haemorrhage in the involved area occurs, establishing infarction This may be followed by central necrosis According to Wagenvoort (14) organization begins after a week at the periphery of the infarct with ultimate scarring of the whole area It could be imagined that, upon disintegration of pulmonary tissue, alkaline phosphatases contained in this tissue enter the circulation Pulmonary tissue, however, shows a relative paucity of alkaline phosphatases Another argument against this hypothesis is the fact that the increase in serum enzyme activity attains a maximum only after a number of days The possibility of a relationship between organization of the infarct by young connective tissue cells and the increase in alkaline phosphatase activity however deserves consideration An argument in favour of this possibility can be found in the abundance of this enzyme activity in young fibroblasts and in the walls of sprouting capillary vessels (4, 6) The interval between the occurrence of the pulmonary infarction and the appearance of the increase in alkaline phosphatase activity is compatible with this theory

It is of importance to point out that our 14 patients all showed radiological pulmonary changes Recently we had the occasion to observe a female patient who a few days after an eye operation, developed acute retrosternal pain with dyspnoea and cyanosis During the course of one day the ECG indicated acute cor pulmonale Pulmonary embolism was diagnosed The subsequent clinical course was characterized by the absence of radiological pulmonary changes — a phenomenon not uncommon in the case of massive pulmonary embolism (7) No increase in alkaline phosphatase activity occurred

A systematic study of the serum alkaline phosphatase activity in relation to the clinical features of thrombo embolic processes and typing of the alkaline phosphatases circulating in the serum under these conditions, are prerequisites for an evaluation of the pathogenesis of this phenomenon

Summary

A transient increase in serum alkaline phosphatase activity was found in 14 patients with pulmonary infarction The maximum value was found 1—3 weeks after the onset of the pulmonary embolism It is improbable that hepatic congestion was the cause of the increased enzyme activity A relationship is suggested between this phenomenon and the organization of the pulmonary lesion by young connective tissue cells

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From the Medical Clinic II (Head Erik Wassén M D) Sahlgren s Hospital University of Göteborg Goteborg Sweden

Treatment of Hypercholesterolemia with Para-aminosalicylic Acid

By

JURI KERSTELL and ALVAR SVANBORG

Riska and collaborators (7) reported in 1955 that the cholesterol level in serum decreased when patients with tuberculosis were treated with para aminosalicylic acid (PAS) Tygstrup and collaborators (9) in 1961 treated 24 patients with an initial cholesterol level between 300 and 450 mg per 100 ml of serum with 12 g PAS per day and observed an average decrease of the cholesterol level of 28 per cent of the initial value

Among the different diseases with an increased level of serum lipids the patients with hereditary essential hypercholesterolemia are known to be very resistent to dietary treatment and earlier used drugs which are known to lower the cholesterol level (5 10) The present report includes the results of treatment with PAS in 7 patients with essential hypercholesterolemia who earlier had been treated with a low fat diet rich in polyunsaturated fatty acids for more than one year The observations show that PAS has a strong effect on cholesterol and phospholipids but did not influence the initially normal triglyceride level in this disease A maximal effect was observed at doses of 8—12 g of PAS per day (0 15—0 20 g/kg body weight) The combined effect of a low fat diet and PAS lowered the plasma cholesterol level on an average with 31 per cent and the phospholipids with 17 per cent of the initial level

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Material and methods

Clinical data from the seven patients are summarized in table I All patients had for more than one year been treated with a low fat diet including around 25 calories per cent from fat and rich in polyunsaturated fatty acids The changes in plasma cholesterol phospholipids and triglycerides induced by this low fat diet were known in four of the patients Patient No 7 had been treated both with a low fat diet and with 2 mg per day of d thyroxine for three years when the treatment with PAS was started PAS was administered orally and initially in three doses of 4 g each either as Na PAS granules or as microgranulized PAS

The methods used in our laboratory for the determination of cholesterol phospho-

TABLE I Clinical data

Patient	Age	Sex	Angina pect	Myocardial infarction	Xanthoma	Xanthelasma	Mean plasma cholesterol value before treatment with diet and PAS (mg/100 ml of plasma)	Duration of PAS treatment (months)
1	41	♀	+			+	491	12
2	41	♀	+		+	+	443	12
3	57	♂	+	+			423	18
4	64	♂	+	?			[1]325	12
5	41	♂				+	368	2
6	53	♂	+	+	+		358	1
7	50	♂	+	+		+	515	1

[1] Value after low fat diet for many years

TABLE II The effect of low fat diet on plasma cholesterol phospholipids and triglycerides

		Cholesterol		Phospholipids		Triglycerides	
		(Mg per 100 ml)	(Decrease, per cent)	(Mg per 100 ml)	(Decrease per cent)	(Mg per 100 ml)	(Decrease, per cent)
Pat no	Treatment	M	M±SE	M	M±SE	M	M±SE
1 2 3 7	–	468	0	331	0	83	0
	Low fat diet	422	8±4	307	6±9	58	31±9

lipids and triglycerides have been described earlier (8) In order to determine if the presence of PAS in the blood influenced the determinations of those lipids 1 and 10 mg per cent of PAS was added to plasma before the lipid analyses and was found not to influence the analyses

Results

The effect of the low fat diet is summarized in table II In table III the further effect of treatment with PAS is shown in five patients treated only with PAS and in one patient treated either with d thyroxine alone or with both d thyroxine and PAS The low fat diet did not significantly lower the level of cholesterol phospholipids or triglycerides

PAS significantly lowered the level of cholesterol ($p < 0.001$) and of phospholipids ($p < 0.01$) but did not in

TABLE III Plasma level of cholesterol phospholipids and triglycerides before and after the treatment with PAS (patient 1—5) or with PAS and d thyroxine (patient 7)

		Cholesterol		Phospholipids		Triglycerides	
		(Mg per 100 ml)	(Decrease per cent)	(Mg per 100 ml)	(Decrease per cent)	(Mg per 100 ml)	(Decrease per cent)
Pat no	Treatment	M	M±SE	M	M±SE	M	M±SE
1—5	Low fat diet	402	0	302	0	66	0
	Low fat diet +PAS	302	25±2	262	12±3	64	3±9
7	Low fat diet	420	0	266	0	39	0
	Low fat diet +d thyroxine	382	9 0	297	+12 0	46	+12 0
	Low fat diet +d thyroxine and PAS	298	22	275	7	42	9

fluence the triglyceride level A cholesterol lowering effect in the same order of magnitude was observed in the patient who had been treated earlier both with a low fat diet and d thyroxine

Fig 1 illustrates the initial effect of PAS on the plasma cholesterol and phospholipids The observed effect in all cases reached a maximum within three weeks of treatment

In two patients who were able to increase the dose of PAS without having side effects, higher doses were tried but did not result in any further lowering of the blood lipids (fig 2) In three of the patients a lowering of the dose to 4 g per day was followed by an increase in the level of cholesterol and phospholipids

PAS induced an average decrease in plasma cholesterol of 25 per cent of the

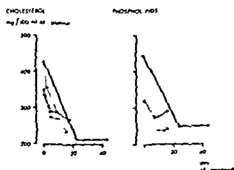


Fig 1 The duration of treatment with 12 g of PAS until maximal cholesterol lowering effect was obtained Dotted lines patients with hypercholesterolemia Continous line a patient with diabetes

level at a low fat diet Thus the combined effect of PAS and the low fat diet was around 30 per cent of the initial level The absolute decrease was greater at initially higher cholesterol levels The effect of PAS calculated as a percentage

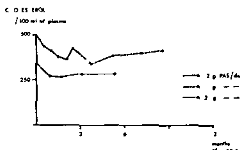


Fig 2 Plasma cholesterol level in two patients during the treatment with various doses of PAS

of the level before treatment was found to be within a range of 20—31 per cent The phospholipids had decreased about 6 per cent during the period with diet only and decreased about 12 per cent further during PAS treatment

Discussion

The mechanism by which PAS lowers cholesterol and phospholipids is not known The results of Tygstrup and collaborators (9) indicate that the acetylation of PAS within the organism is essential for the effect An increase of fat excretion by the stools during PAS treatment was also reported

Salicylic acid is known to lower the lipolysis of triglycerides in adipose tissue (2), but if PAS has a similar effect seems to be unknown The present observation that the level of triglycerides was less influenced than that of cholesterol and phospholipids in plasma indicates that the plasma lipid lowering effect does not mediate via a lowered rate of the fatty acid release in adipose tissue, which should have been followed by a lowered triglyceride synthesis (4) Salicylic acid also influences the binding of thyroxine to plasma proteins but PAS seems to be less active in this respect (11) On the contrary, PAS can induce hypothyroidism (1, 6) In the present material one of the patients for three years had been treated with the highest dose of d thyroxine tolerated The effect of PAS was found to be in the same order of magnitude as in the other cases

Earlier reports (3) and preliminary observations in our laboratory indicate that PAS also has a pronounced effect on plasma lipids in diabetes In one patient with juvenile diabetes, who had been treated with a low fat diet for several months, the level of cholesterol and phospholipid in plasma was still high, about 500 mg/100 ml of plasma and 400 mg/100 ml of plasma respectively The treatment with PAS induced a further decrease in the cholesterol concentration of 46 per cent of the level on a low fat diet (fig 1)

Summary

Seven patients with essential hypercholesterolemia were treated with 4—12 g of PAS orally per day Before the PAS treatment all patients had received a low fat diet rich in polyunsaturated fatty acids for at least one year The decrease of plasma cholesterol averaged 100 mg/100 ml or 25 per cent and that of plasma phospholipids 40 mg/100 ml or 12 per cent of the level obtained with a low fat diet The maximal effect was obtained within three weeks of treatment and at doses of 8—12 g of PAS per day (0 15—0 20 g/kg body weight) The initially normal triglyceride levels were not significantly altered

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From the Department of Internal Med cine (Head W J Bruins Slot M D) Zu der Z ekenhu s and the Central Patholog cal Laborator (Head H E Schornagel M D) the Munic pal Hosp tals Rotterdam The Netherlands

Coincidence of Adrenal Atrophy, Haemolytic Anaemia and Hyperthyroidism in a Patient Followed by Sudden Death

By

C H L KLAASSEN C K W VAN DOMMELEN and J A M VAN UNNIK

In the follow ng paper data will be presented on a patient suffering from adrenal cortical atrophy as well as idiopathic haemolytic anaemia and hyperthyroidism

Rarity of this triad leads us to report this case and the post mortem findings

Case report

F s adm on On Apr l 2nd 1957 a 46-year old marr ed oman was admitted to our ward because of collapse in the out pat ent department during rad ological ex am nat n She as be ng n est gated for recurr ng abdom nal pa ns and easy fat gua b l ty For a fortn ght she had vomited repeatedly At phys cal exam nat on p g mentat on of skin and skinfolds was found Mucosal pigmentat on was absent Her he ght was 161 cm we ght (af er three da s trea ment as 52 kg O herwise there ere no ph s cal abnormal t es Blood pressure was 105 80 m m Hg pulse rate 100 m n regular temperature 37 7 C.

Laborator data haemoglob n 16 g % haematocrit 49 % WBC 7 500 mm³ eosinophils 400 mm³ urine no prote n reduct on absent urob l nogen trace sediment negat ve

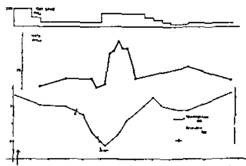
Fast ng blood sugar 85 mg % serum electrolytes sod um 138 meq l potass um 5 2 meq l serum urea 100 mg % A d agnos s of adrenal insufficiency was made and therapy was started ith D C A. i m hydrocort sone and infus ons of 0 9 % NaCl The d agnos s was supported by the absence of eos nopen a after ACTH i m Intracutaneous tubercul n test was negat e Flat abdom nal X ray sho ed no abnormal calcificat ons

Successful ma nten nce therapy was D C A pellets n the rectus sheath tw ce a year nd t ice dail 12 1 2 mg cort sone The pat ent as d scharged we ght 55 8 kg on May 28th 1957 th he d agnos s of Adc son s d sease probabl caused by ad renal atrophy

Se ond ad miss on On No ember 9 1957 she was readm tted the ma n compla nts be ng om t ng dizz ness and jaund ce She had been well un l October 28 1957 At ph s cal exam na on e found jaund ce e ght 55 kg blood pressure 115 85 Pulse

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Presen address Roode Kruis Z ekenhu s, Vondellaan 13 Be erw jk The Ne herlands



F g 1 The apy re culocy es haemoglob n and b l rub n le els dur ng a per od of haemolyt c anaem a

rate 120 Temperature 38 3 C Pa nful r ght upper abdom nal quadrant No further abnormal t es Labo atory data Hb 14 4 g °₀ WBC 5 000/mm³ Urine prote n absent glucose negat ve urob l nogen strongly pos t ve b l rub n absent Serum b l rub n 3 7 mg °₀ d rect react on negat ve No ele ation of alkal ne phosphatase ammon um sulphate turb d ty and thymol turb d ty Bromsul phale n test normal SGOT and SGPT norm l X ray of gallbladder no abnor mal t es The jaund ce d sappeared after a week the temperature became normal and the pat ent was dis harged Probable d ag nos s cholecystit s

Th rd adn on Two eeks later on December 17 h 1957 the pat ent was aga n adm tted because of om t ng jaund ce and fever Phys cal exam nat on re ealed no new data blood pressure 80 55 pulse 104 regular temperature 38 4 C Haemoglob n 13 2 g °₀ WBC 12 000 Ur ne prote n fa ntly pos t e no reduct on u ob l nogen strongly pos t ve Sed ment conta ned blood (menstruat on) Serum b l rub n 3 9 mg °₀ d rect react on negative The pat ent was transferred to the surg cal department where laparotomy as performed Result normal gall bladder normal append x During her stay n the hosp tal a new syn drome de eloped cons st ng of fe er jaun d ce anaem a and we ght loss Ser m b l ru b n rose to 4 4 mg °₀ d rectly negat ve RBC decreased to 2 5 m ll on/mm³ and ret culocytes rose to 55/1 000 Temperature dur ng th s ep sode was bet veen 39 C and 40 C dur ng a fortn ght

Osmot c frag l ty of the red blood cells was normal The d rect Coombs test negat e The sternal marrow sho ed act e normoblast c erythropoes s and leucopo es s The stercob l nogen excret on in the faeces as 705 mg/3 days

Serum ron was 144 mg °₀ th no un sat rated b nd ng capac ty Dur ng th s pe r od of fe er cort sone dosage was increased to 150 mg per day and the pat ent recovered (fig 1) Cort sone dosage was then gradually decreased to 25 mg tw ce da ly Ten weeks after adm ss on the pat ent was d scharged n good cond t on w th the d agnos s of Add son s d sease compl cated by an ep sode of unexplained haemolyt c anaem a We ght was 47 4 kg She was treated as an outpat ent dur ng the next n neteen months She ga ned we ght during the first months n February she we ghed 55 5 kg After that her we ght d d not change sign ficantly

Fou th adn ιss on On October 2nd 1959 she vas readmitted for implantat on of D C A pellets She had been nervous for t o months appet te had been excellent but yet she had lost some we ght The thyro d vas enlarged there was a bru t over it The sk n was warm and mo st The pulse rate was 110 regular there was a fine tremor of the hands Blood pressure 160/90 temperature normal We ght 5? 2 kg one week later 51 2 kg Laboratory d ta haemoglob n 14 3 g °₀ WBC 8 700 ret culocytes 6/1 000 ur ne normal serum b l rub n 1 8 mg °₀ P B I 8 1 m crogram °₀ B M R + 35 °₀ one week later + 31 °₀ Of a tracer dose of ¹³¹J 75 °₀ was excre ed n the ur ne in 48 hours The pat ent was treated with carb mazole and prom nal Pulse rate dropped to about 78 she ga ned some we ght

She left the hosp tal on Oc ober ?3rd 1959 w th the (add t onal d agnos s of hyperthyro d sm

Treatment was cont nued n the out pat ent department On No ember 2nd 1959 B M R was + 20 °₀ on December 21st B M R as — 1? °₀ and we ght as

60 5 kg Carbimazole dosage was decreased gradually and this therapy was stopped on September 22nd 1960 On October 10th 1960 B M R was + 13 % weight was 64 8 kg

Final admission She was again admitted on November 26th 1960 There had been vomiting and abdominal pain jaundice had reappeared Physical examination revealed jaundice thyroid slightly enlarged tenderness in the left lower quadrant Blood pressure 135/90 temperature 38 0 C pulse rate 108 regular weight 60 5 kg Laboratory data haemoglobin 15 6 g % WBC 7 900/mm³ reticulocytes 4/1 000 urine trace of protein no glucose urobilin strongly positive many leucocytes in the sediment

Blood serum bilirubin 2 6 mg % sodium 137, potassium 4 5 chloride 97 bicarbonate 25 1 meq/l

Urea 66 mg % iron 113, unsaturated binding capacity 175 microgram % direct Coombs test negative L E test negative Bacteriological examination 3 blood cultures remained sterile From the urine a growth of E coli and S faecalis was obtained

During the first week temperature was elevated bilirubin rose to 4 5 mg % stercobilinogen excretion in the faeces was 478 mg in three days With a diagnosis of haemolytic anaemia in view we gave her 30 mg prednisone daily in addition to her usual dose of cortisone (50 mg daily) and fludrocortisone 0 1 mg daily

Temperature became normal, vomiting stopped serum bilirubin fell to 0 8 mg % stercobilinogen excretion fell to 68 mg per three days

On December 11th she *weighed 54 5 kg* on January 13th weight was 57 5 kg

Prednisone dosage was gradually lowered and this treatment was stopped on January 7 1961 Temperature rose to subfebrile haemoglobin fell to 11 g % reticulocytes were 30 1 000 Stercobilinogen excretion was 364 mg per three days bilirubin was 1 7 mg % General condition remained fair but on January 25th 1961 the patient developed rigors and died suddenly The blood culture *showed no growth.*



Fig 2 Toxic goitre with tall columnar epithelium scanty colloid and lymphoid tissue (A 8147 Haematoxylin azophloxin × 22)



Fig 3 Very small suprarenal gland almost totally replaced by connective tissue The overall structure is visible (A 8147 Haematoxylin azophloxin × 30)

Autopsy was performed 18 hours after death Significant findings were The thyroid measured 6 × 6 × 4 cm it looked normal Histological examination showed high follicular epithelium scanty and thin colloid Some lymphoid tissue was present One *small necrotic area was found* (fig 2)

The liver weighed 940 g No iron pigment was found The gallbladder contained dark green bile and no stones The spleen weighed 305 g On histological examination some phagocytosis of erythrocytes was found and some erythropoiesis but no iron pigment

No adrenal glands could be found In the suprarenal fat small structures were found 0 7 × 0 7 × 0 3 cm

Microscopical examinations showed the presence in one of these structures of a blood

vessel that could have been the vena centralis or the adrenal gland, it was surrounded by connective tissue with three outgrowths, measuring 0 15 by 0 05 cm and imitating in shape an adrenal gland (fig 3) Clumps of rather big cells were seen in them, resembling cells of the adrenal medulla, and also a few cells were found which were thought to be atypical cortical cells as described by Schaberg (5) The hypophysis was normal

Conclusion adrenal atrophy Graves disease, splenomegaly

Discussion

Combined disease of the thyroid and adrenal glands, without pituitary disease, is not rare De Cock et al (4) found in a group of 21 cases of Addison's disease, 2 cases of hyperthyroidism, it was not clear which disease had first arisen Soffer et al (6) and Bruno et al (3) both saw further instances of combined Graves and Addison s disease In 17 cases Addison's disease came first and in 8 cases hyperthyroidism was the first disease (1, 2)

One might suppose that thyrotoxicosis could exhaust the adrenal glands, but no theory can explain why adrenal atrophy might lead to thyrotoxicosis

Combined haemolytic anaemia and hyperthyroidism has been noted before in one case (7)

We think that our patient suffered from combined adrenal atrophy haemolytic anaemia and hyperthyroidism Necropsy findings proved the first diagnosis Arguments in favour of a diagnosis of haemolytic anaemia were enlarged spleen with erythrocyto-phagocytosis, elevated faecal stercobilinogen excretion during periods of fever and fall in haemoglobin values, with return to normal during treatment with prednisone

Necropsy findings agreed with a diagnosis of hyperthyroidism and laboratory data was characteristic as was the response to treatment

The final period and her sudden death seemed inexplicable to us at the time, however, we now come to a possible explanation Relapse of hyperthyroidism is common, when treatment with carbimazole is stopped too soon We think our patient had such a relapse, which increased her need for cortisone, as possibly also did the fever A dose of 50 mg of cortisone may have been far too low under these circumstances, and she may have died in acute Addisonian crisis

Summary

A woman was admitted to hospital five times in the course of four years with successive diagnoses of Addison's disease cholecystitis?, haemolytic anaemia? hyperthyroidism, and haemolytic crisis with sudden death (possibly in Addisonian crisis)

At necropsy extremely atrophic adrenals were found and also the other findings were in agreement with the clinical diagnoses

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From the Department of Medicine Lofoten sykehus Lofoten Norway

Cardiac Arrhythmia Associated with Cheyne-Stokes Breathing

By

T Barkve and P Stavem

With Cheyne Stokes breathing the pulse rate may vary with the different phases of respiration In cases where the periodic breathing is caused by increased intracranial pressure the slower pulse usually occurs during the apnea When on the other hand, the Cheyne Stokes breathing is caused by heart disease or cerebral arteriosclerosis the slower pulse usually occurs during the respiratory phase (2)

In cardiac patients with Cheyne Stokes breathing the slow pulse during the respiratory phase can be caused by sinus slowing more rarely by displacement of the pacemaker to the atrioventricular node, second or third degree atrioventicular block or ventricular standstill sometimes causing Stokes Adams attacks (2) All these changes in cardiac rhythm can result from vagal effect In most cases atropine has when tried abolished the cardiac arrhythmia without changing the periodic breathing A cyclic increase in vagal tone is therefore thought by many to be the cause of the various arrhythmias during the respiratory phase of the periodic breathing (1) This cyclic increase in vagal tone might be caused by hypoxia acting upon chemoreceptors of the carotid body the hypoxia being most pronounced early in the respiratory phase of the periodic breathing (1) Most of these patients have been digitalized which fits well with the fact that digitalis increases the sensitivity of the carotid sinus reflex

We have recently observed a patient with Cheyne Stokes breathing who exhibited several of these arrhythmias at the same time

Case report

A woman 80 years of age was admitted to the medical department of Lofoten sykehus September 16 1965 because of increased drowsiness and confusion She had been known to have hypertension at least since 1962 She had been admitted to hospital with myocardial infarction in January 1964 and later that year had developed congestive failure treated with salt restrictions digitoxin and thiazide diuretics Towards the end of 1964 she developed diabetes mellitus which was treated with diet and chlorpropamide

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During the last few years the relatives had become aware of a somewhat defective memory

On admission the patient was somewhat undernourished She was drowsy and gave quite irrational answers to questions There was Cheyne Stokes breathing with brady cardia at a rate of 32 per minute during the respiratory phase and tachycardia at a rate of 105 per minute during apnea No rales were heard over the lungs There was no pitting edema of the legs but moderate edema over the sacrum There was no facial asymmetry, and the patient was able to move all 4 extremities There was no Babinski reflex

Laboratory examinations Blood sugar on admission was 230 mg/100 ml (glucose oxidase method) The plasma Ketostix test was negative Lumbar puncture gave clear, colorless fluid without any increase in leucocytes or protein contents ECG showed signs of myocardial infarction

Course The patient became more soporous and comatous and died 3 days after ad mission She received small doses of insulin, and her fasting blood sugar was normal 2 days after admission

Post mortem examination (Microscopic ex amination except brain by Dr F Skjorten Neuropathological examination by Dr A Torvik Ullevål sykehus Oslo)

The patient was 152 cm weight 52 kg The heart weighed 480 g The ramus an terior of the left coronary artery was occluded 3 cm from its beginning Section through the myocardium revealed a fibrotic area in the anterioseptal region Microscopic examination showed broad strands of fibrous tissue separating bundles of somewhat hypertrophic muscle fibers The nuclei as well as the striation were well preserved with no sign of necroses or recent circula tory disturbance The right lung weighed 410 g the left lung 320 g In the right lower lobe was a hemorrhagic infarct The liver weighed 1 500 g The cut section had a *nutmeg appearance Microscopic examina* tion showed marked congestion and necrosis of centrilobular liver cells The kidneys weighed 350 g together The surface was slightly granular, the capsule was thin and stripped easily The cortex was about 6 mm Microscopic examination showed essentially normal renal tissue The brain was rather small, and both frontal lobes showed some degree of atrophy The vessels at the base showed numerous atheromatous patches without any total occlusion Sec tions failed to reveal any gross lesions in the cerebrum, brain stem or cerebellum There were no signs of hemorrhages or infarcts *Microscopic examination of Bodian stained* sections revealed numerous senile plaques and neurofibrillary changes in the hippo campal region, and also in other parts of the cerebral cortex There was no evidence of anoxic damage

Studies of the arrhythmia

A continuous electrocardiographic trac ing was made in relation to the breathing during 5 whole cycles of the Cheyne Stokes breathing During early apnea each P wave was followed by a QRS complex with a PQ interval of about 0 18 sec and the heart rate was about 105 per minute During the later part of the apnea and the early part of the respiratory phase the atrial rate fell off to less than 50 per minute A QRS complex followed each P wave with a PQ interval of about 0 20 until the atrial rate was less than 50

In one of our recorded cycles the P waves disappeared altogether, leaving a succession of evenly spaced QRS complexes with a rate of 35 per minute (fig 1) We interpreted this as atrio ventricular nodal rhythm After about 15 sec of atrioventricular nodal rhythm P waves could be made out before each QRS complex, and the heart rate start ed to increase The P waves were at

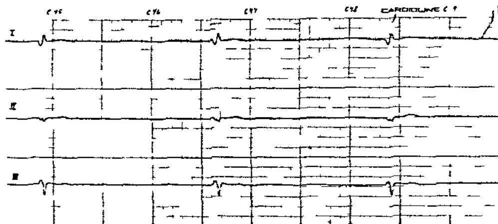


Fig 1 Leads I II and III showing uniformly spaced QRS complexes with a rate of about 35 per minute No P waves are discernible Interpreted as atrioventricular nodal rhythm

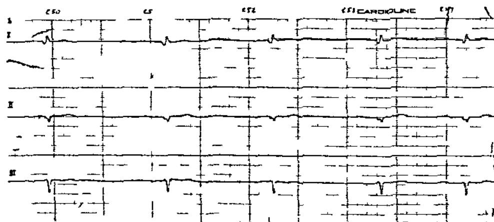


Fig 2 Leads I II and III showing QRS complexes preceded by P waves which at first are inverted and have a short PQ interval This part of the continuous ECG is a direct continuation of the part shown in fig 1

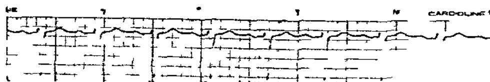


Fig 3 Lead V_4 showing sinus tachycardia during apnea of Cheyne Stokes breathing

first inverted in lead III and the PQ interval was short but the P wave and PQ interval then assumed a more normal appearance (fig 2)

In the 4 other cycles the P waves were discernible throughout and the lowest atrial rate in each cycle was about 42 per minute This lowest atrial rate was

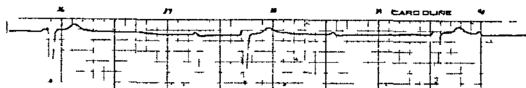


Fig 4 Lead V₂ showing complete atrioventricular block during the resp ratory phase of Cheyne Stokes breathing This part of the continuous ECG is taken 30 sec after the part shown in f g 3

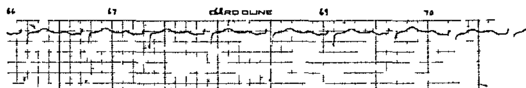


Fig 5 Lead V₂ showing sinus tachycard a during apnea of Cheyne Stokes breathing Th s part is taken 30 sec after the part shown in fig 4

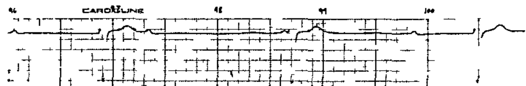
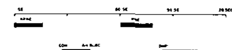
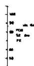


Fig 6 Lead V₂ showing complete atrioventricular block during the resp ratory pl ase of Cheyne Stokes breath ng This part is taken 30 sec after the part shown in f g 5





F g 7 The time of the apnea the t me of the total atrioventricular block and the atrial rate for 2 cycles of the Cheyne Stokes breath ng The time scale in seconds corresponds to the time scale on the ECG shown in figs 3—6

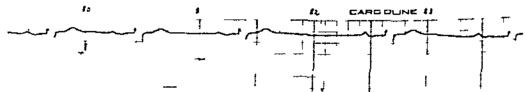


Fig 8 Lead V₂ showing the abrupt trans t on to the more peaked P waves as the complete atrioventricular block is approached



Fig 9 Lead V₂ showing the abrupt transit on away from the peaked P waves at the po nt when the complete atrioventricular block has just worn off

accompanied by a complete atrioventricular block lasting for 16—26 sec The ventricular pacemaker must have been located in the atrioventricular node or in the bundle of His, as the QRS complexes were identical to the ones resulting from the sinus impulses (figs 3—6) The ventricular pacemaker initiated QRS complexes at a rate of about 32 per minute and each time took over without any ventricular standstill the longest RR interval being 2 sec The transition from sinus rhythm to idioventricular rhythm was rather abrupt, with no 2nd degree atrioventricular block the last conducted P wave had a PQ interval of 0 22—0 25 sec

The time of apnea, the time of the total atrioventricular block, and the atrial rate were correlated for two cycles of the periodic breathing (fig 7) The time scale in seconds in fig 7 corresponded with the time on the ECG strips pictured in figs 3—6 Figs 3—6 revealed the P waves in lead V to be somewhat more peaked during pronounced bradycardia than during rapid sinus rhythm The transition to the peaked P waves when the total block was approached (fig 8) and the transition away from the peaked P waves when the total block had just worn off (fig 9) occurred rather abruptly

Discussion

Our patient developed a pronounced sinus bradycardia during the later part of the apnea and the early part of the respiratory phase, the atrial rate falling from 105 to below 50 per minute Sinus slowing during this phase of Cheyne Stokes breathing in cardiac or arteriosclerotic disease, has been reported several times before although in many instances far less pronounced (2)

In one of our registered cycles the P waves disappeared after the atrial rate had fallen below 50 per minute leaving a succession of evenly spaced QRS complexes with a rate of 35 per minute We interpreted this as a shift to atrio ventricular nodal pacemaker Towards the middle of the respiratory phase a P wave could again be made out before each QRS complex and the heart rate started to increase The first few P waves were inverted in lead III, and the PQ interval was short This we interpreted as due either to a more rapid retrograde spread to the atria or else to the pacemaker having moved to a higher level in the atrioventricular node Resnik and Lathrop (4) and later a few others, have described a shift to atrioventricular nodal rhythm in this phase of Cheyne Stokes breathing

In 4 of our registered cycles the atrial rate fell to about 42 during the early part of the respiratory phase and remained at this rate for a while At the same time a total atrioventricular block appeared, and a center in the bindle of His or in the atrioventricular node took over immediately without any ventricular standstill Similar cases have been described several times previously in the literature in many instances also with ventricular standstill which at times caused Stokes Adams attacks (2 3)

In our 4 registered cycles with total atrioventricular block the P waves in V_2 were more peaked during pro

nounced bradycardia than during rapid sinus rhythm, but the PQ interval remained about the same The peaked P waves started before and lasted longer than the total atrioventricular block We interpreted this change to more peaked P waves in V as probably due to a change from sinus pacemaker to another atrial one, e g coronary sinus pacemaker Steele and Anthony described a patient who also showed a change in form of the P waves during the bradycardia of the respiratory phase (5) In their patient the P waves during bradycardia frequently were inverted in lead I, and they concluded "It seems probable that marked slowing of the normal pacemaker allows a lower portion of the auricle or a portion of the auriculoventricular system to take over this function Matthews and Wood also described changes in the P waves during sinus bradycardia of the respiratory phase, suggestive of a shift in the pacemaker (2)

Summary

A patient who was maintained on digitoxin because of arteriosclerotic heart disease with congestive failure developed signs of cerebrovascular accident without any hemiplegia There was Cheyne Stokes breathing, and various arrhythmias were registered late in the apnea and early in the respiratory phase

1 Marked sinus slowing, the atrial rate falling from 105 to 42 per minute

2 Shift of pacemaker from the sinus node to the atrioventricular node

3 Shift from sinus node to a lower atrial pacemaker (probably coronary sinus), together with development of complete atrioventricular block

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From Medical Department F (Head L Korsgaard Christensen M D) Gentofte Hospital
Copenhagen Denmark

The Androsterone-etiocholanolone Excretion Ratio in Hyper- and Hypothyroidism

By

L Skovsted, J Molholm Hansen M Kristensen and
L Korsgaard Christensen

Androsterone and etiocholanolone are the two major end products of androgen metabolism In androsterone the hydrogen atom attached to C 5 is in the α position whereas the same atom in etiocholanolone is in the β position Apart from this structural difference the two metabolites are identical Their major androgen precursors are testosterone Δ^4 androstene 3,17 dione and dehydroisoandrosterone these substances being secreted by the testes and the adrenal gland

In 1956 Bradlow et al (1) made the observation that administration of triiodothyronine increased the production of androsterone in euthyroid as well as in myxedematous subjects This interesting question of the thyroid androgen interrelationship in euthyroid, myxedematous and hyperthyroid subjects was later studied by the same group of workers (3 4) The excretions of androsterone and of etiocholanolone derived from endogenous secreted androgens were determined by chromatographic methods They also studied the metabolism of intravenously administered testosterone-4 ^{14}C The excretion ratio of labeled androsterone to labeled etiocholanolone was found to agree very closely with the excretion ratio of the same two metabolites as determined by the chemical procedure

In a control group of seven subjects androsterone and etiocholanolone were found to represent on the average 40 per cent and 60 per cent respectively of the total excretion of these two substances *Five subjects with myxedema* showed a pronounced decrease in androsterone formation to about 15 per cent of the total amount whereas 5 hyperthyroid patients had an increase in androsterone production to 50 per cent or more

The present study is primarily concerned with the development of a comparatively easy procedure for the determination of the ratio of labeled andro-

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sterone to labeled etiocholanolone excreted after the intravenous administration of testosterone 4 ^{14}C The clinical applicability of this procedure was tested in a group of patients with hypo and hypermetabolism

Material

The material consisted of 17 myxedematous patients, 22 cases of thyrotoxicosis and a control group of 23 euthyroid normal subjects A clinical diagnosis of myxedema or of thyrotoxicosis was confirmed by a determination of the BMR the PBI the 4 and 24 hour uptake of radioiodine and the $PB^{131}I$ The patients in the control group had a normal 4 and 24 hour ^{131}I uptake test and a normal $PB^{131}I$

Methods

Chemicals

Androsterone etiocholanolone and testosterone — were crystalline products obtained from Sigma Chemical Company

The testosterone-4 ^{14}C was obtained from New England Nuclear Corp One microcurie of the radioactive material was diluted with one ml of a benzene solution containing one mg of non labeled testosterone and kept frozen until use

For liquid scintillation counting the following counting solution was used 3 g of *PPO* 0 5 g of *POPOP* and 12 5 g of Thixcin were suspended in 500 ml of toluene and the mixture was homogenised for 3 min with a tissue homogenizer Nitrogen was bubbled through the suspension for 10 min before the gel suspension was ready for use

Infusion of radioactive testosterone and collection of urine

A volume of the radioactive solution corresponding to one microcurie testosterone was evaporated to dryness under a stream of nitrogen and transferred with a few ml of ethanol to 100 ml of isotonic saline which was then given to the subject by infusion over a period of 40—50 minutes The infusion bottle was weighed before and after the administration and an aliquot of the infusion solution was analysed for radioactivity in order to calculate the precise dose administered Urine was collected for the next 24 hours in 6 hour fractions and stored below 0 C Another 24 hour urine collection was used for a 17 ketosteroid determination

Hydrolysis and extraction of urine

An aliquot of the first 6-hour urine fraction was hydrolysed and extracted according to the method of Johnsen (5) From 60 to 250 ml were used depending on the total volume of this 6 hour fraction The urine was adjusted to pH 0 8 by addition of 40 % sulfuric acid, transferred to a round bottomed boiling flask and half a volume of benzene was added The urine was boiled under reflux for 15 min and subsequently cooled Finally the benzene layer was separated This extraction procedure was repeated twice with the remaining water layer The second time the urine was readjusted to pH 0 8 benzene was added and the mixture was boiled for 30 min Prior to the third extraction procedure the urine was adjusted to pH 0 2 and after addition of the benzene it was boiled for one hour The combined benzene extracts were washed three times with 1/10 volume 2 N sodium hydroxide and then repeatedly with 1/10 volume distilled water until neutral and finally dried with anhydrous sodium *sulphate The extract* was filtered and then evaporated to dryness under reduced pressure The dried residue was dissolved in ethanol and transferred to a small test tube and evaporated to dryness above an infra lamp and under a stream of nitrogen The residue was finally dissolved in one ml of ethanol and was now ready for separation by thin layer chromatography

Thin layer chromatography

About 20 μl hydrolysate was applied to a 20 20 cm glass plate coated with the adsorbent silica gel G On the same plate a

standard mixture of testosterone androsterone and etiocholanolone (about 20 μg of each) was applied together with the sample and also in a separate spot for identification The chromatogram was developed in benzene ethyl acetate (1 1 v/v) (2) for about 45—50 min (solvent front 14 cm) After completion of the run the plate was dried at room temperature and then placed in a chromatography tank containing iodine vapor until colored spots appeared The three standard reference zones and the corresponding zones of the urine extract were immediately marked since the color will disappear on standing Now the zones of the sample corresponding to testosterone androsterone and etiocholanolone were carefully removed from the plate by means of two nickel spatulas and transferred directly to the counting tubes for radioactivity assay

Liquid scintillation counting

The radioactivity was measured by a scintillation counter (Isotope Developments Limited) using a gel suspension counting technique The limitation imposed by the solubility of the samples in the toluene — P P O solution has been overcome in this technique by suspending a solid sample in a solvent system containing the gelling agent called Thixcin About 5 ml of this Thixcin gel counting solution was transferred by a syringe pipette to the counting tubes containing the steroids adsorbed to the silica gel and before counting the solid particles were suspended very carefully into the gel by stirring with a small glass rod The activity of the urine hydrolysate and that of an aliquot of the infusion solution was also counted in the gel uspension

Reproducibility

To test the reproducibility of the method six 100 ml aliquots of the same urine fraction were carried through the procedure As seen from table I the values for ratio of androsterone to the combined amount of androsterone and etiocholanolone in the 6 assays agreed very satisfactorily

TABLE I Reproducibility of the method

Subject	$\frac{A}{A+E}$ (%)	$\frac{E}{A+E}$ (%)
E P	19 4	80 6
	19 5	80 5
	17 9	82 1
	19 1	80 9
	19 3	80 7
	20 5	79 5
Mean ± S D	19 3 ± 0 8	80 7 ± 0 8

TABLE II The excretion ratio determined twice with a 72 hour interval in each of 3 different subjects

Subject	$\frac{A}{A+E}$ (%)	$\frac{E}{A+E}$ (%)
N L.	80 0	20 0
	80 3	19 7
N L.	20 2	79 8
	19 8	80 2
C.L.	23 8	76 2
	23 7	76 3

To find out whether the ratio $\frac{A}{A+E}$ would be the same in different urine fractions collected during the first 24 hours after the infusion of testosterone 5 fractions constituting a 24 hour urine collection from one subject were analysed and it was found that the ratio was unchanged during a 24 hour period Consequently it was decided to analyse only the first 6 hours fraction throughout this study

Table II shows the results from an experiment in which the $\frac{A}{A+E}$ ratio was determined twice in 3 different subjects Testosterone-4 ^{14}C was administered twice to each subject at an interval of 72 hours and

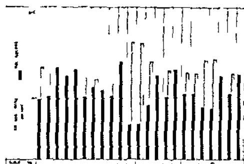


Fig 1 The etiocholanolone androsterone excretion ratio in normal subjects

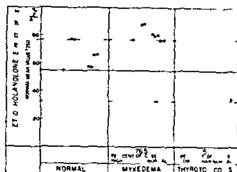


Fig 4 Normal range of $\frac{E}{E + A}$ and the values found in myxedematous and thyrotoxic patients

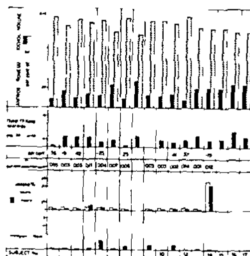


Fig 2 The excretion ratio in myxedematous patients

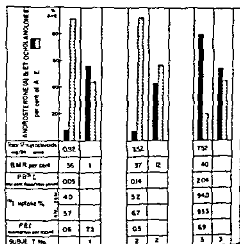


Fig 5 Effect of treatment on the etiocholanolone androsterone excretion ratio

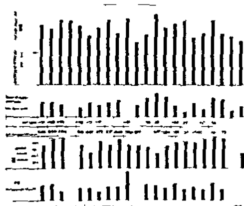


Fig 3 The excretion ratio in thyrotoxic patients

urine from the first 6 hours after the infusion was collected and analysed As shown in table II the results from the 2 separate experiments agreed very satisfactorily

It should further be mentioned that with the combined TLC and radioassay a recovery of about 84 % of the radioactivity added was obtained

Results

The determinations of the subjects in the control group are shown in fig 1 The average value of $\frac{E}{E + A}$ was found to be 54 6 % The normal range

(mean ± 2 S D) of the etiocholanolone fraction was 32 5—76 9 % (fig 4)

The results obtained in the group of hypothyroid patients are shown in fig 2 In all these patients the etiocholanolone fraction was greatly increased Converse ly a decrease of the etiocholanolone fraction was found in the hyperthyroid patients (fig 3) From fig 4 appears that 76 5 % of the myxedema cases excreted an etiocholanolone fraction above the upper normal range whereas only 59 1 % of the thyrotoxic patients showed values below the lower normal range

The results shown in fig 5 clearly demonstrate the change in the etio cholanolone androsterone ratio following treatment of two myxedematous and of one thyrotoxic patient

Some few euthyroid patients with a decreased BMR were found to have a normal etiocholanolone androsterone ex cretion ratio

Discussion

The principal purpose of the present work has been to develop a comparative ly simple and accurate method for the determination of the etiocholanolone androsterone excretion ratio From the preceding results it may be seen that the procedure described is quite satis factory In a few of our cases the deter mination has been of practical diagnostic value as far as the diagnosis of myxedema was concerned Evidently however this procedure may be too complicated to find a more general use as a laboratory procedure for the diagnosis of thyroid disorders

In fig 2 was shown that an increase in the etiocholanolone fraction was observ ed in all the cases of myxedema studied This increase is pronounced even in cases with a very moderate decrease in BMR The opposite trend was observed in thyrotoxicosis (fig 3) The increase of the androsterone fraction is however not proportional to the increase in the metabolic rate (compare for example subjects nos 15 and 16)

It is well known that androsterone and etiocholanolone are structurally identical except for the orientation of the hydrogen atom at the C 5 position which is α in androsterone and β in etiocholanolone Testosterone is oxi dized via the 17 ketonic pathway to androstenedione and then reduced by 5 α and 5 β reductases to androsterone and etiocholanolone respectively Kap pas et al (6) made the very interesting observation that the 5 β compound (etiocholanolone) has a pyrogenic effect in man With this fact in mind a relative increase in etiocholanolone production might be supposed to be benefical to subjects who are myxedematous and hypometabolic (3 8) Conversely it may evidently be considered desirable for the hypermetabolic thyrotoxic pa tient to be able to form more andro sterone and less of the pyrogenic etio cholanolone

The important experiments of Mc Guire and Tomkins (7 8) may possibly provide the biochemical explanation of the above findings They observed that administration of thyroxine to rats caused an increase in the liver 5 α microsomal reductases which will favour the formation of androsterone over that of et ocholanolone

Summary

A procedure for the determination of the etiocholanolone-androsterone ratio in urine is described The normal range of etiocholanolone in per cent of the total amount of the two compounds was found to be 32 5—76 9

An excretion ratio above the upper normal range was found in 76 5 per cent of myxedema cases In a group of thyrotoxic patients 59 1 per cent showed values below the lower normal range

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From the Departments of Internal Medicine at Karolinska Sjukhuset (Head H Lagerlof M D) and at Serafimerlasarettet (Head G Biorck M D) and King Gustaf V Research Institute (Head G Birke M D) Stockholm Sweden

Serum Lipids, Intravenous Glucose Tolerance and Their Interrelation Studied in Ischaemic Cardiovascular Disease

By

LARS A CARLSON and FREDRIK WAHLBERG

In so called ischaemic disease a group of which examples are myocardial infarction angina pectoris and intermittent claudication the serum lipids as well as the glucose tolerance are often abnormal The concentration of cholesterol and/or triglycerides in serum may be elevated (2 3 5 8 13) and the oral (6 19 21) as well as the intravenous (23) glucose tolerance may be reduced Furthermore several reports have demonstrated various derangements of carbohydrate metabolism in different kinds of hyperlipoproteinaemia such as hypercholesterolaemia (22) and hypertriglyceridaemia (1 12 14 15 17)

This investigation was undertaken to study the relationship between lipid and carbohydrate metabolism in ischaemic diseases as revealed by determination of fasting concentrations of cholesterol and triglycerides in serum and by the intravenous glucose tolerance test (IVGTT) Furthermore an estimate of the frequency of abnormalities of these parameters of lipid and carbohydrate metabolism in ischaemic disease was obtained

Material and methods

For the present study *ischaemic disease* was restricted to myocardial infarction angina pectoris and intermittent claudication All patients with *myocardial infarction* had been hospitalized during their acute illness and presented at least 2 of the 3 following criteria a clinical history ECG and or transaminase (GOT GPT) changes suggestive of acute myocardial infarction *Angina pectoris* and *intermittent claudication* were diagnosed from a typical history and the latter diagnosis was also confirmed by oscillometry of the legs Accordingly *100 male and 22 females were selected* their serum lipids and IVGTT being unknown The male group contained

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TABLE I Age levels of cholesterol and triglycerides in serum and k value for the intravenous glucose tolerance test in 100 men and 22 women with ischaemic disease

Age (yrs)	Cholesterol (mg/100 ml)	Triglycerides (mmole/l)	k (%/min)
Men			
Mean value			
59	290	2 27	1 14
Range			
36–78	163 432	0 60 12 0	0 40 2 98
Women			
Mean value			
61	294	1 88	1 32
Range			
45 76	213 380	0 83 5 92	0 65 2 66

64 survivors of myocardial infarction 24 patients with angina pectoris and 12 patients with intermittent claudication Corresponding figures for the women were 11 4 and 7 Another 20 male patients were selected on account of the presence of *ischaemic disease as well as hyperlipoproteinaemia* the mean age being 53 years Ten of them had survived one or more myocardial infarctions 7 had angina pectoris and 3 had intermittent claudication

The material consists of patients who were accessible for study from October 1961 until June 1964 All patients were ambulatory in good general condition at the time of the study and there was a minimum interval of 1 month since any previous acute myocardial infarction They were on their ordinary diets and in no case had any dietary prescriptions been made aimed at reducing the blood lipid levels or the weight No patient had a history suggesting diabetes or glucosuria or other condition known to affect carbohydrate metabolism

The patients reported in the morning to the laboratory after fasting overnight After a rest in the recumbent position for at least 15 min , capillary blood samples in duplicate or triplicate were as subsequently drawn from the earlobes for glucose determination A needle was then inserted into an antecubital vein and blood withdrawn for lipid analysis whereupon the intravenous glucose tolerance test (IVGTT) was started by injection of 25 g of glucose in a 60 % aqueous solution through the same needle Zero time was set at the end of the injection Capillary blood samples were taken after 10 and 20 min and from then on at every fifth minute until 60 min with the samples at 20 and 60 min in duplicate (23) The blood glucose values between 20 and 60 min form an apparently straight line when plotted against time in a semilogarithmic system (23) By extrapolation of this line the k value for the disappearance of blood glucose in per cent per minute was estimated by graphical methods

The blood withdrawn for lipid analysis was allowed to clot at room temperature for one hour and the serum was then immediately separated off by centrifugation The sera were kept at — 14° C until analysed Serum lipids were determined as described earlier (7) cholesterol essentially according to Sperry Webb (20) and triglycerides according to Carlson (10) Blood glucose was determined with glucose oxidase according to Marks (18)

The upper normal' limits for serum cholesterol and triglycerides have been set at 322 mg/100 ml and 2 20 mmole/l respectively on the basis of previously established normal values for healthy men in Stockholm (7) Values for k of 0 90 and lower have been called diabetic 0 91—1 10 borderline and 1 11 and higher normal (23)

Statistical calculations were performed by testing differences between means and distributions according to Wilcoxon and correlations according to Spearman the parameters studied not being normally distributed

Results

The male and the female groups of patients selected on account of ischaemic disease are characterized with regard to age, serum lipid values and k values in table I These variables exhibit a wide range of values and their means do not differ significantly on comparison between the men and the women

	Serum lipids					
	Cholesterol	Normal	Elevated	Elevated	Normal	Total
	Triglyceride	Normal	Normal	Elevated	Elevated	
IV Glucose Tolerance	Normal	19	6	5	14	44
	Borderline	14	1	3	5	23
	Diabetic	17	3	7	6	33
	Total	50	10	15	25	100

Fig 1 Distribution of 100 male patients with ischaemic disease according to the occurrence of normal or abnormal serum lipids and IVGTT The upper normal limit for cholesterol was 322 mg/100 ml and for triglycerides 2 2 mmole/l (7) A borderline IVGTT was defined as k = 0 91 -1 10 per minute and a diabetic as k < 0 90 (23)

Frequency of serum lipid and glucose tolerance abnormalities

The 100 males selected due to the presence of ischaemic disease were grouped according to their serum lipid pattern and k value as shown in fig 1 Fifty of them had some plasma lipid abnormality and 56 had borderline or diabetic IVGTT The most frequent lipid abnormality was elevation of only the serum triglycerides which occurred in 25 men Elevation of only serum cholesterol occurred in 10 men and elevation of both these lipid fractions in 15 A diabetic IVGTT was encountered in 33 men and a borderline one in 23

Of the 50 men with normal plasma lipids 31 had borderline or diabetic IVGTT, which means that *only 19 men of the 100 studied had both serum lipids and IVGTT within normal limits*

Of the 22 women with ischaemic disease 11 had some plasma lipid abnormality and 9 had borderline or diabetic IVGTT (Abnormality according to normal values defined for men (7) In a recent unpublished study (11) we have seen that healthy men and women in Stockholm have fairly similar serum cholesterol levels while the women have significantly lower triglyceride levels)

Of the 11 women with normal plasma lipids 5 had diabetic or borderline IVGT, which leaves 6 of the 22 women with normal serum lipids and IVGT

Hyperlipoproteinaemia and IVGTT

There were no significant differences in the distributions of the k values between the 50 male patients with normal serum lipids and the total group of 50 patients with hyperlipoproteinaemia, nor among the 3 groups with hyperlipoproteinaemia (fig 1) Nor were any significant differences obtained on separate comparison of the patients with normal serum lipids and either the ones with elevated cholesterol only or those with elevation of both cholesterol and triglycerides However the k values of the group with elevated triglycerides only were significantly higher ($P < 0.05$) than those of the group with normal lipids the mean k values being 1 30 and 1 03, respectively

The male patients with ischaemic disease and known hyperlipoprotein

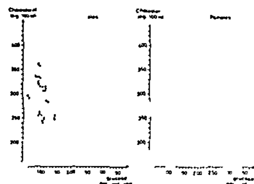


Fig 2 Relationship between the concentration of serum cholesterol and the IVGTT (k value) for men (left) and women (right) ● = patients selected on account of ischaemic disease ○ = patients selected on account of hyperlipoproteinaemia

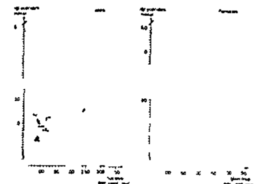


Fig 3 Relationship between the concentration of serum triglycerides and the IVGTT (k value) for men (left) and women (right) ● = patients selected on account of ischaemic disease ○ = patients selected on account of hyperlipoproteinaemia The two highest values for the men were 7 5 and 12 0 mmole/l

aemia were added to the material above since the result of an increase of the hyperlipaemic groups was considered to be of interest in this context Of these 20 patients, 7 had elevation of only cholesterol, 7 elevations of cholesterol and triglycerides, and 6 elevated triglycerides only In this pooled group no significant changes occurred of the above mentioned results with regard to the relationship between hyperlipoproteinaemia and IVGTT

Serum cholesterol level and IVGTT

The serum cholesterol and the k values are plotted in fig 2 for the men and the women It is obvious that no relationship existed between serum cholesterol and k value for the men, and statistical analysis showed that $R < 0.16$ ($P > > 0.05$)

For the women, on the contrary, the k values increased significantly with increasing concentration of cholesterol in serum The correlation coefficient for the concentration of cholesterol in serum and the k value was $R = 0.69$ ($P < 0.01$)

To ascertain whether age had any effect on these results, the men and the women were divided into subgroups by ages above and below the mean age For the males there was still no correlation between serum cholesterol and k value either for the 48 men below 59 years ($R < 0.24$, $P > 0.05$) or for the 52 men above this age ($R < 0.23$, $P > 0.05$) For the 10 women younger than 61 years the rank correlation coefficient between serum cholesterol and k values was $R = 0.68$ ($P < 0.05$) and for the 12 older women the corresponding figure was R 0.71 ($P < < 0.02$)

Serum triglyceride level and IVGTT

The serum triglycerides and the k values are given for the men and the women in fig 3 No correlation between these two parameters can be seen either for the men ($R < 0.17$, $P > 0.05$) or for the women ($R < 0.36$, $P > 0.05$) Nor

were any correlations obtained after subdivision of the material by age as described above for cholesterol

Serum lipids and fasting blood glucose levels

The relationship between the fasting blood glucose level and the k value is shown in fig 4 for the males and the females For both sexes there was a significant negative correlation, for the males R = −0 37 (P < 0 001) and for the females R = −0 74 (P < 0 001) As there was only a low grade correlation between these two variables for the men, it was of interest to study whether the fasting level of blood glucose correlated better to the serum lipids than the k value

The relationships between the fasting blood glucose levels and the serum lipids for the men and the women are shown in figs 5 and 6 The only significant correlation — a negative one — was between cholesterol and blood glucose for the women, in whom high cholesterol levels were associated with a low concentration of blood glucose (R = −0 57, P < 0 05) This was not unexpected as they had shown fairly strong correlations on the one hand between k value and cholesterol level and on the other hand between k value and fasting blood-glucose level

Serum lipids IVGTT, and weight index

Obesity has been found to be associated with derangements of lipid and carbohydrate metabolism This prompted us to study the relation between weight index obtained as weight of patient/normal weight (16) and serum cholesterol triglycerides and k value as is

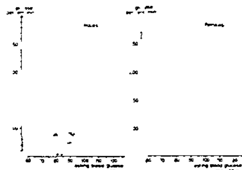


Fig 4 Relationship between the fasting level of blood glucose and the IVGTT (k value) in 100 men (left) and 22 women (right) with ischaemic disease

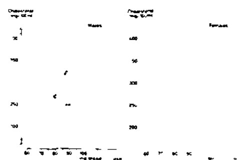


Fig 5 Relationship between the fasting level of blood glucose and the concentration of serum cholesterol in 100 men (left) and 22 women (right) with ischaemic disease

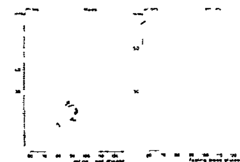


Fig 6 Relationship between the fasting level of blood glucose and the concentration of serum triglycerides in 100 men (left) and 22 women (right) with ischaemic disease The two highest values for the men were 7 5 and 12 0 mmole l

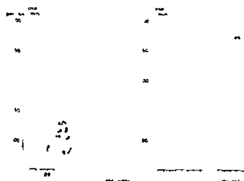


Fig 7 Relationship between the IVGTT (k value) and the weight index in 100 men (left) and 22 women (right) with ischaemic disease



Fig 8 Relationship between the concentration of serum cholesterol and the weight index in 100 men (left and 22 women (right with ischaemic disease

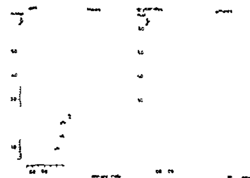


Fig 9 Relationship between the concentration of serum triglycerides and the weight index in 100 men (left) and 22 women (right) with ischaemic disease The two highest values for the men were 7 5 and 12 0 mmole l

illustrated in figs 7 8 and 9 The triglyceride level tended to increase with increasing weight index in men ($R = 0.30$ $P < 0.01$) otherwise no significant correlations emerged

To evaluate the effect of a high weight index on the relation between serum cholesterol triglycerides and k value the 23 men and the 7 women with a weight index above 1 15 were selected No correlations between the serum lipid levels and the k value were thereby obtained

Discussion

Several studies on patients with ischaemic disease have dealt with serum lipids and/or glucose tolerance However, few studies of ischaemic disease are available in which serum lipids and glucose tolerance have been studied and interrelated in the same patient

In the present study it is of interest to note that about as many patients had some kind of serum lipid abnormality as had an abnormally decreased glucose tolerance and that only around 20 per cent of the patients were normal with regard to *both* serum lipid levels and glucose tolerance In the context of serum lipids triglyceride abnormalities were more common than cholesterol abnormalities Similar results have been reported in earlier studies of ischaemic diseases (2 3 5) It is also interesting to note that the frequency of abnormal serum lipids as well as of abnormal glucose tolerance is similar to that obtained by Carlson (8) and Wahlberg (23) in earlier unrelated studies Therefore the results of the present study are thought to be representative of those

obtained in similarly selected patients with ischaemic disease

The existence of interrelationships between lipid and carbohydrate metabolism in tissues is well documented Therefore it is not surprising that several authors have looked for certain relationships between serum lipids and glucose tolerance as these parameters might reflect derangements of metabolism on a cellular plane Several studies have been performed on patients selected on account of major plasma abnormalities such as hypercholesterolaemia, hypertriglyceridaemia, and overt diabetes The occurrence of impaired glucose tolerance in idiopathic hyperlipaemia was described by Lewer et al in 1954 (17) Adlersberg and Wang reported in 1955 on 5 patients with idiopathic hyperlipaemia with mild diabetes mellitus (1) In 20 patients with idiopathic hypercholesterolaemia the majority of whom had been selected due to the presence of vascular disease Waddell et al found an abnormal oral glucose tolerance in 18 (22) In 14 subjects with marked fasting hypertriglyceridaemia Kane et al found that only 3 had normal oral glucose tolerance (14) In patients selected on account of diabetes it is now well established that hypertriglyceridaemia is the dominating serum lipid abnormality especially when the control of carbohydrate metabolism is poor (9)

In this study however, we could not demonstrate any relationship between high serum lipid levels and low glucose tolerance Moreover there was no increase in the frequency of abnormal IVGTTs even in the patients with the most excessive lipid abnormalities For example 2 of the 5 male patients with cholesterol above 400 mg per 100 ml plasma had a k value below 1 10, while the same figures for the patients with cholesterol below 220 were 3 of 6 Similarly 2 out of 5 male patients with triglycerides above 5 mmole l had a k value below 1 10 while the same figures in patients with triglycerides below 1 mmole/l were 5 out of 10 Apparently the frequency of abnormal k values does not vary with the serum levels of either cholesterol or triglycerides in the present material of patients with ischaemic disease Similar results were obtained by Reaven et al (19) They found that a group of 41 patients with myocardial infarction had a reduced oral glucose tolerance when compared to a control group of patients without coronary artery disease The levels of cholesterol and triglycerides in plasma were also elevated However they could not find any statistically significant relationship between carbohydrate intolerance and hyperlipaemia Their results are thus on a par with our findings in the present material of 100 patients with ischaemic disease The fact that in our patients the IVGTT was indeed higher in the group with elevated serum triglycerides only than in the group with normal serum lipids should of course not be taken as a reflection of the general behaviour of the IVGTT in hypertriglyceridaemia In the interpretation of our results it should be kept in mind that the intravenous and not the oral glucose tolerance was used in our patients who furthermore were selected on account of ischaemic disease

Furthermore in the women the IVGT increased with increasing cholesterol levels This contradicts any supposed association between hypercholesterol aemia and decreased glucose tolerance at least in patients with ischaemic disease

Summary

The fasting levels of cholesterol and triglycerides in serum and the intra venous glucose tolerance were deter mined in 100 male and 22 female patients selected on account of ischaemic disease (myocardial infarction angina pectoris intermittent claudication)

Fifty of the men had some kind of serum lipid abnormality hypertrigly ceridaemia being more common than hypercholesterolaemia A borderline or abnormal glucose tolerance was found in 56 of the men Only 19 men had *both serum lipids and glucose tolerance* within normal limits The figures for the small female group were similar

In the male group there was no tendency for the glucose tolerance to decrease with increasing levels of either cholesterol or triglycerides in serum On the contrary the glucose tolerance was on the average *higher* in the group with elevated triglycerides only than in the group with normal lipids

In the female group the glucose tolerance was positively correlated to the serum cholesterol levels the correlation coefficient between these two parameters being statistically significant

There was a slight positive significant correlation between the "weight index and the triglyceride level for men

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From the Department of Rheumatology (Head B Olhagen M D) Karolinska sjukhuset and King Gustaf V Research Institute (Head G Birke M D) Stockholm Sweden

Separation and Immunologic Characterization of Hog Intrinsic Factor in Comparison with Human Intrinsic Factor

By

Ragnhild Gullberg

It was previously shown in gel diffusion experiments combined with autoradiography that only a minor vitamin B_{12}-binding component in hog intrinsic factor (IF) preparations cross reacted with rabbit antiserum to human IF preparations and reacted with serum from pernicious anemia (p a) patients treated with hog IF orally (16) The main component showed immunologic similarity to a B_{12} binding component in hog bile and liver (16)

In the present work recycling gel filtration (7 22 26) has been used to separate the two components so as to enable further characterization and determination of their relationship to IF

Material and methods

^{60}Co-labeled cyanocobalamin (AB Atomenergi Stockholm) usually mixed with unlabeled cyanocobalamin to give a specific activity of 0 5 mC per mg was added to the IF solutions according to the B_{12} binding capacity estimated by a dialysis method (27)

Submitted for publication February 4 1966

Hog IF concentrate W ES 947 (Lederle Pearl River N Y) was kindly supplied by Dr L. Ellenbogen

Hog bile and serum were obtained from newly killed hogs

Purified IF related B_{12} binder S (10) from human gastric juice to which ^{57}Co-cyanocobalamin (spec activ about 20 mC/mg) had been added was kindly supplied by Drs R Grasbeck and K Simons Helsinki

A concentrate of the IF related B_{12} binder of normal human gastric juice was prepared as follows The gastric juice was neutralized in situ (13) with sodium phosphate buffer (pH 7 2 μ 0 2) and the mixture was batch filtered through DEAE-cellulose (Whatman) equilibrated (25) with the same kind of buffer as was used for the intragastric neutralization

The eluate was concentrated by ultra filtration through a collodion membrane (Membranfilter Gesellschaft Gottingen)

Purified human gamma globulin and albumin (AB Kabi Stockholm) The commercial albumin solution contained sodium acetyl tryptophan giving absorption at 254 mμ

Blue dextran 2 000 (AB Pharmacia Uppsala) with a molecular size > 200 000 and excluded from the Sephadex G 200 gel

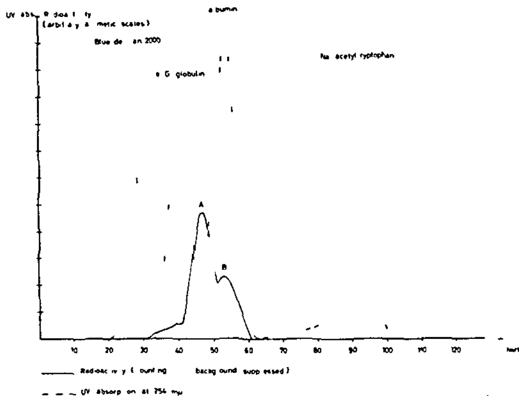


Fig 1 Gel filtration on a Sephadex G 200 column (3 × 117 cm) of hog IF concentrate WLS 912 (Lederle) labeled with [illegible]Co-cyanocobalamin and reference substances. Eluant buffer was 0.1 M Tris HCl in 1 M NaCl pH 8.0 containing 0.02 % sodium azide. The radioactive component A was eluted between human gamma G globulin and albumin. The component B had the same elution volume as human albumin

Antisera produced by immunization of rabbits with human or hog IF preparations according to an earlier described technique (15, 16). In a similar way antiserum to hog gall bladder bile was produced, 2 ml bile being used for each injection.

Sera from p. a. patients with precipitating antibodies to a B_{12} binding component in human or hog IF preparations (17).

Recycling gel filtration (7, 22, 26) was performed on Sephadex G 200 (AB Pharmacia). A Tris-NaCl buffer was used (0.1 M Tris-HCL in 1 M NaCl, pH 8.0) containing 0.02 % sodium azide. The gel bed in the plexiglass column was 3 × 117 cm. Tygon tubes (Sigmamotor Inc., Middleport, N.Y.) with an inner diameter of 1/32 inch were connected to the applicators at the top and the bottom of the column. A Sigmamotor pump T 8 was supplied with an extra transmission gear to make it suitable for a low flow rate of about 10 ml/hour. In the eluate the absorption at 254 mμ was measured by an Uvicord absorptiometer (LKB-produkter AB, Stockholm). The radioactivity was measured in a coil of the Tygon tube (1.5 ml) placed against a scintillation detector, Ekco type N 559 A with a crystal N 566 A, coupled to an Ekco type 522 ratemeter via a type N 640 pulse amplifier. The high voltage and gain were adjusted so that the photopeaks of [illegible]Co were registered. The outputs from the ratemeter and Uvicord were registered in separate channels of a Westronics Model LD 11 A two-channel recorder. When sufficient separation of the peaks had been obtained, the eluate was switched to a drop collector. As the change of fractions in the

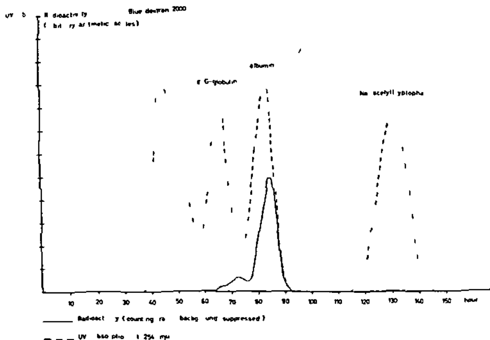


Fig 2 Gel filtration on Sephadex G 200 of a human IF concentrate prepared by batch filtration through DEAE-cellulose (see text) to which ^{60}Co-cyanocobalamin had been added The main radioactive component was eluted with the peak slightly after that of human albumin

collector was marked on the recorder it was easy to take out those fractions which contained the peaks and to measure their radioactivity more accurately in a well crystal recorder For advanced technical advice and assistance the author is indebted to L O Plantin first research engineer King Gustaf V Research Institute

For recycling gel filtration 50 mg hog IF preparation WES with ^{60}Co-B_{12} was applied to the column An example of an experiment with bleeding and recycling of the column is demonstrated in fig 3 The collected fractions were concentrated by ultrafiltration

All preparatory procedures were performed at + 4 C

Paper electrophoresis was performed in sodium borate buffer pH 9 0 and ionic strength 0 12 and in Veronal buffer pH 8 6 and ionic strength 0 1 The paper strips were autoradiographed and then stained with amide black as described in an earlier work (14)

The *immunoprecipitin reactions* were studied by a micro version (34) of the Ouchterlony technique (24) with use of autoradiography to visualize the reactions of B_{12} binding substances (15 16)

The *Schilling tests* were kindly performed by dr P Reizenstein The oral dose contained 0 5 μg cyanocobalamin and the 48-hour urinary excretion of the absorbed radioactive B_{12} was measured (28)

Results

In gel filtration experiments on Sephadex G 200 the IF preparation was applied to the column together with reference substances The major B_{12} binding component (A) in the hog IF

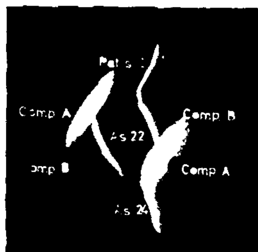


Fig 6

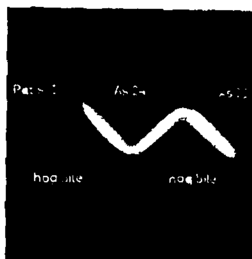


Fig 7

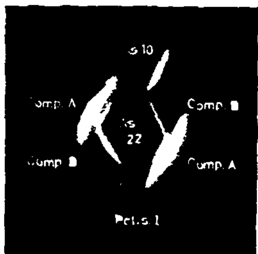


Fig 8

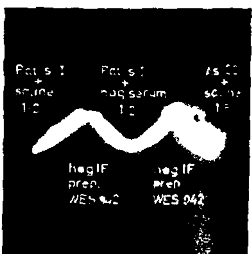


Fig 9

these sera having been shown to react with a B_{12} binder in hog II preparations in previous works (16 17) figs 6 and 8) The patients antibodies that reacted with component B did not react with any B_{12} binder in hog bile fig 7) and did not cross react with human II (16, 17) They were not absorbed by pre incubation of patients serum with hog serum or bile (figs 9 and 10 In reactions with rabbit antiserum to hog II preparation WES 912 it could be shown that the precipitin line of component B fused into a line caused by a fraction in component A fig 8 Grasbeck s purified II related B_{12} binder S 10 from human gastric juice gave a precipitate with sera from some of the p a patients these sera having previously been found to react with a B_{12}

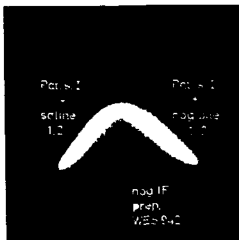


Fig 10

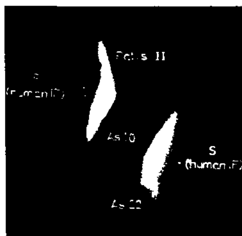


Fig 11

Figs 6—11 Show photographs of autoradiograms of immunodiffusion experiments The reagents *shown in the figures are listed below For explanations see text*
Antigens Hog IF concentrate Lederle WES 942 (hog IF prep WES 942) Component A (comp A) and component B (comp B) separated from the hog IF concentrate WES 942 by gel filtration Grasbeck s purified IF related B_{12} binder S from human gastric juice (S) Hog bile
Antisera Rabbit antiserum to electrophoretically purified human IF (As 10) Rabbit antiserum to hog IF concentrate WES 942 (As 22) Rabbit antiserum to hog bile (As 24)
Sera from p a patients Pats I was serum from a patient which contained antibodies to hog IF Pats II was serum from a patient which contained antibodies to human IF Mixtures of Pats I with hog serum or bile or saline (1 2) preincubated 30 min at room temperature before application were used in figs. 9 and 10

binder in whole human gastric juice (12 16, 17) (fig 11) S or a fraction of S reacted with rabbit antiserum to hog IF concentrate WES 942 (12) (fig 11)

Discussion

The physicochemical and immunological characterization of IF and other B_{12}-binding substances and the determination of their biologic activities by in vivo and *in vitro experiments are hampered* by difficulties in preparing the B_{12} binders in their pure and native form (8 9) However comparative studies of B_{12} binding components labeled with radioactive vitamin B_{12} and separated from each other could give information about some of their properties

Hog gastric or duodenal mucosa contains both IF active and inactive B_{12} binders (8 9) Many workers have attempted to isolate IF (8 9) The most potent hog IF B_{12} complex has been prepared by Bromer et al (3) and Grasbeck et al (11)

In the present study the hog IF concentrate WES 942 was used as starting material and for separation of *two components which bind the bulk* of added radioactive cyanocoblamin the technique of recycling gel filtration on Sephadex G 200 was used (7 22 26) (fig 3)

The molecular size of the larger component called A was found to be between that of human gamma G globulin

and that of albumin as judged from gel filtration experiments with reference substances on Sephadex G 200 (fig 1) The smaller component, B, was found to have a molecular size similar to human albumin (fig 1) and slightly larger than the IF-related B_{12}-binder of human gastric juice, neutralized in situ, (fig 2) Only component B possessed IF activity according to Schilling tests in p a patients (table I) Various molecular weights of hog IF — from about 5 000 to about 130,000 — have been reported (8 9) The present results could agree with those of Holdsworth (19), who calculated the molecular weight of hog IF to be about 55 000 from *ultracentrifugation results* and with those of Gräsbeck et al (10) who estimated the molecular weight of human IF as about 60 000 from gel filtration experiments on Sephadex G 200 However, the molecular size determined by gel filtration does not always correlate with the molecular weight determined by ultracentrifugation (22) The discrepant results concerning the molecular weight of IF are probably mainly caused by difficulties in preparing IF in a pure and well defined form Split products of IF may still possess IF activity (8) Furthermore IF molecules in different degradation stages might from complexes with each other or with other proteins It has even been assumed that IF may be a prosthetic group which could attach to various mucoproteins (8)

The component A could correspond to the main component of Andresen et al (1) and to Holdsworth's fraction 1 (19), *which were inactive* The larger molecular size of the inactive component A is also in agreement with Holdsworth's work

Holdsworth's IF active fraction 2 had a slower electrophoretic mobility to the anode than the inactive fraction 1 in the buffer used (19) On the other hand, Barlow et al (2) reported a correlation between a fast anodically migrating B_{12} binder and the IF activity of various hog IF preparations In the present study it was noticed that the relative electrophoretic mobilities of the two separated components were very much influenced by the kind of buffer used (figs 4 and 5) In sodium borate buffer pH 9 0 the component B migrated more slowly than A to the anode, whereas it *migrated slightly faster than component* A in Veronal buffer pH 8 6 This could be due to a difference in the carbohydrate content of the two substances (19)

Differences in antigenic structure of the two B_{12} binders were demonstrated by the immunodiffusion technique combined with autoradiography A B_{12} binder in hog bile showed immunologic similarity to component A but not to the IF active component B (fig 6) Thus no support was obtained for the theory that IF might be present in hog bile (8 16) but it must be emphasized that the IF activity was determined in a heterologous species only Earlier results did not indicate the presence of any IF related B_{12} binder in human bile (16 31) However this does not exclude the possibility that bile might contain breakdown products of IF

Only the IF active component B *showed immunologic* similarity to human IF (fig 8) Component B was also

the only one reacting with antibodies in sera from hog IF treated patients (fig 8)

It is plausible that a similarity in structure of human and hog IF explains why hog IF is — at least temporarily — active in man A similar antigenic structure was demonstrated by cross-reacting antibodies produced in rabbits (8, 9 16) (figs 8 11) It is presumed that an organ specific and immunologically similar part of the human and hog IF molecules is of importance for the IF function defined as the specific ability of IF to permit a sufficient intestinal absorption of vitamin B_{12} under physiologic conditions (8)

Little is known about the passage of IF B_{12} complexes or other B_{12} binders through cellular membranes and about the metabolism of IF, in e g the intestinal wall There is no direct evidence that IF is released into the body from the intestinal mucosa cells However, supposing that parts of an IF B_{12}-complex pass through the intestinal wall, it may be difficult to demonstrate such absorbed IF fragments The metabolites of IF might have lost IF-activity and they might also have lost other characteristics of IF e g parts of its antigenic sites The major portion of absorbed B_{12} has been shown to be transported with the portal blood This has been thought to contradict the idea that a relatively large IF B_{12}-complex would be absorbed from the gut However, some of the absorbed B_{12} passes with the lymph and some remains in the intestinal wall for a long time Absorbed and injected B_{12} have been found to have different plasma clearances Experimental data on the active B_{12} absorption have recently been reported or reviewed by several authors (4, 6, 8, 9 18, 29, 35) It is conceivable that relatively small quantities of absorbed IF fragments would be sufficient to cause an immune response

The hypothesis that fragments of an IF B_{12} complex are absorbed from the gut is put forward in an attempt to explain the distinctive pattern of the precipitin reactions of human and hog IF with antibodies in patients sera in comparison with those in experimentally produced antisera (16 17) Patients antibodies to hog IF could be produced in a similar way as antibodies to other ingested proteins (33) It is however, notable that precipitating antibodies reacting with hog IF could be demonstrated in the majority of hog IF-treated patients with pernicious anemia, and often in patients with other diagnoses who have received hog IF orally (17) In contrast no antibodies to component A or to other proteins in hog IF preparations were found (17), but it must be pointed out that the method used was more sensitive in demonstrating reactions with components to which radioactive vitamin B_{12} had been bound than reactions with unlabeled proteins Furthermore, the hog IF antibodies in patients serum were not absorbed by non-gastric hog proteins e g hog serum or bile (figs 9 and 10) This accords with the results of Lowenstein et al 23) Therefore it is postulated that either antigenic structures of hog IF are absorbed from the intestine by a mechanism specific to IF or there is some other cause for the relatively strong antigenicity of hog IF after oral administra

tion Schwartz (30) showed that hog IF with a high B_{12} saturation more rapidly caused refractoriness to oral hog IF than the same preparation with a low B_{12} content Vitamin B_{12} is thought to stabilize the IF-molecule or at least the B_{12} binding part of it, making it more resistant to proteolysis (8), and to make it more efficiently bound to surface receptors of the intestine (4 18, 32) The hog IF might be incompletely or differently metabolized in human intestinal cells if the active enzymes are species specific However, provided that there is no significant difference in the digestion and metabolism of hog IF and autologous IF in the human gastro-*intestinal tract, the antibody response* to ingested hog IF would be an indirect evidence for the absorption of human IF-structures from the gut in man It could be presumed that man is immunologically tolerant to such absorbed autologous IF fragments

The immunologic tolerance to an absorbed form of autologous IF would explain why hog IF antibodies in patients sera do not cross react with human IF (16 17 30) Only that part of the absorbed hog IF molecule which differs from an absorbed part of human IF would induce antibody production in man Thus the hog IF antibodies in patients sera and the cross reacting antibodies in rabbit antisera to human IF, which were reacting with component B, could be directed to different antigenic sites The former antibodies would then react with species specific determinants of the part of hog IF that is absorbed *from the gut and the latter* with organ specific determinants of the hog IF molecule It is even possible that parts of human and hog IF molecules, e g the postulated absorbed fragments are so similar that a slight difference in antigenic structure could be detected with antibodies produced in man but not with such produced in the rabbit (20) If some part of human and hog IF were identical, it would be possible to prepare a fragment of hog IF devoid of determinants antigenic to man

As antibodies to human IF were not more often found in hog IF treated patients (17), no support was obtained for the idea that immunization to ingested hog IF would induce auto-antibody production to IF in man

The auto-antibodies to human IF (5, 8, 9, 17, 33) are supposed to be produced by a different mechanism They would be directed not to the postulated absorbed part of a human IF B_{12} complex, to which man is thought to be immunologically tolerant, but to other parts of the human IF, which could reach antibody producing cells when gastric mucosa is undergoing cellular destruction Auto-antibodies to IF have been found only in p a patients It has been suggested that pernicious anemia would be an organ specific auto-immune disease (5 33)

These theories on the antibody production to human or hog IF in man could agree with the effects of the IF antibodies on the IF mediated B_{12} absorption (8 9) Serum antibodies to hog IF in p a patients have been found to be correlated with refractoriness to oral hog IF according to some investigations (17 23 30), *but they do not diminish* the effect of human IF in p a patients

On the other hand, serum antibodies to human IF in p a patients do not cause refractoriness either to human or to hog IF Those antibodies to IF could be shown to suppress the B_{12} absorption only if the IF is given orally, mixed with the antibody containing serum in a so called in vivo inhibition test, in which they cross react with hog IF (8, 9)

Another hypothesis on the refractoriness to oral hog IF has been presented by Kaplan et al (21) They immunized two p a patients by injections of a purified hog IF concentrate Serum antibodies were produced that gave positive immunologic in vitro tests i e electrophoretic retention test with a hog IF concentrate and (in one of two patients) positive in vivo inhibition test with hog IF but the patients immunized did not become refractory to oral hog IF In the same study, sera from five patients refractory to hog IF gave with one exception negative in vitro tests with the hog IF concentrate "Since there is no correlation in the individual patient between the degree of systemic sensitation to hog intrinsic factor and the ability of the latter to enhance vitamin B_{12} absorption it is concluded that the refractory state must be determined by local factors within the gastrointestinal tract It is possible however to put alternative interpretations on *their results* *Firstly the presence of* B_{12} binders other than IF in the hog IF-preparations would be of importance for the immune response and for the in vitro tests used but probably not for the in vivo inhibition test and might thus have caused erroneous conclusions concerning IF Secondly, the route of the antigen administration, i e parenteral or oral might be of significance in still another way than the one proposed by Kaplan et al If the IF is given by injections the antibodies produced might preferably be directed to other antigenic sites of the molecule than to those of the part absorbed from the gut This could also agree with their finding that the patient s antibodies to injected hog IF, despite a positive in vivo inhibition test did not cause refractoriness to hog IF In these respects those antibodies were analogous to the spontaneously occurring antibodies to human IF in p a patients Nevertheless local antibodies to ingested hog IF might be of importance for the refractory state It could however, be assumed that the circulating and local antibodies would be produced to the same part of the IF molecule viz antigenic structures of a hog IF B_{12} complex absorbed from the intestine

Summary

Two B_{12} binding components in complex with radioactive cyanocobalamin were separated by recycling gel filtration from the hog intrinsic factor concentrate WES 942 Their molecular size was estimated by comparison with reference substances The larger component with a molecular size between that of human gamma G globulin and that of albumin showed similarity in antigenic structure to a B_{12} binder in hog bile in immunodiffusion experiments combined with autoradiography The smaller component with a molecular size similar to albumin and slightly larger than human intrinsic

factor showed antigenic similarity to human intrinsic factor and reacted with sera from patients treated with hog intrinsic factor orally Only this smaller component possessed intrinsic factor activity according to Schilling tests in patients with pernicious anemia The results of immunologic comparison of human and hog intrinsic factor are discussed with reference to theories on the immune response in man to different antigenic structures of human and hog intrinsic factor

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From the Department of Medicine Karolinska Institutet at Serafimerlasarettet (Head G Biorck M D) the Departments of Surgery (Head J Adams-Ray M D) Urology (Head G Giertz M D) and Pathology (Head B Thorell, M D), Karolinska sjukhuset and King Gustaf V Research Institute (Head G Birke M D) Stockholm Sweden

Renal Carcinoma

An Attempt to Correlate Symptoms and Findings with the Histopathologic Picture

By

I E Bottiger, C Blanck and T von Schreeb

Renal carcinoma, because of its peculiar symptomatology, has attracted much interest It is possible clinically to separate two rather distinct groups of cases, *one* consisting of the classical urologic patients with hematuria as the dominating symptom *the other* comprising the later described non urologic patients, with vague symptoms — often difficult to interpret — such as weight loss fatigue anemia and elevated erythrocyte sedimentation rate (2 12) Most pathologists have accepted the view that from a morphological point of view there exist two different types of renal carcinoma cells those with clear and those with granular cytoplasm respectively Cahill (7) stated that the former type had a clinical picture separating them from the latter and Foot (11) that clear cell carcinoma carried a better prognosis than the granular cell type Bottiger (5) published a report where biochemical findings were correlated to the histological type of the tumor

This is an extension of that latter study, reporting findings in 46 new cases together with those of the 54 previously described The findings in the new series corroborate the previous results and the two series, with a total of 100 patients with renal carcinoma are here discussed together Our results support the view that the symptoms and findings in renal carcinoma depend on the histological type of the tumor

Material

The material is composed solely of cases of renal carcinoma with a definite patho-anatomical diagnosis after operation All instances of transitional cell carcinoma sarcoma etc have been excluded The patients were treated at different hospitals in and around Stockholm during the period 1958—64 Letters were sent and personal calls

Submitted for publication February 7 1966

factor showed antigenic similarity to human intrinsic factor and reacted with sera from patients treated with hog intrinsic factor orally. Only this smaller component possessed intrinsic factor activity according to Schilling tests in patients with pernicious anemia. The results of immunologic comparison of human and hog intrinsic factor are discussed with reference to theories on the immune response in man to different antigenic structures of human and hog intrinsic factor.

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TABLE I Symptomatology—initial symptoms

	One single symptom[1]	Several symptoms	
Hematuria macroscopic	45		50
Systemic symptoms[1]			
Weight loss		11	
Fatigue	[1]19	14	54
Fever		10	
Elevated ESR		19	
Symptoms from metastases	13		13
Local pain caused by tumor	10		14
Tumor encountered unexpectedly	8		8
Palpable tumor (felt by patient)	3		3
Disorders of micturition	2		7
	100		149

The first column represents an attempt to determine the single symptom that prompted the patient to seek medical advice Is was found impo sible however to distinguish between the symptoms within the systemic group

TABLE II Predominant cell type

	With metastases	Without metastases	Total
Clear-cell carcinoma	14	23	37
Granular cell carcinoma	32	31	63
	46	54	100

Malignancy grading (according to Arner et al (1))

	I	IIA	IIB	III	
Clear cell carcinoma	1	16	17	2	36
Granular cell carcinoma	3	18	31	9	61
					[1]97

[1] *In three cases insufficient material for grading*

Men predominate (male/female ratio 1 8/1) the mean age is 60 years the youngest patient a 28 year-old woman

The *initial symptoms* are given in table I It may be seen that although hema turia is the most common single symptom the systemic manifestations added together come next and are far more com mon than the other symptoms in the clas sic triad of renal carcinoma (hematuria pain and palpable tumor) This triad may be left out of further discussions

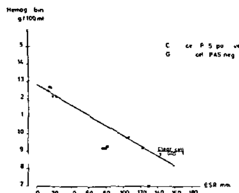


Fig 4 Correlation between ESR and hemoglobin in patients without metastases with clear cell (n = 21) and granular cell (n = 23) tumors respectively (Time matched values were missing in 6 cases) The regression line and equation are given for the patients with clear cell tumors

macroscopic and 10 microscopic hematuria *This means that 40 patients were entirely free from all kinds of hematuria*

Table IV gives the way in which the diagnosis was reached

Mean pre-operative values in patients without apparent metastases (see Discussion) for *ESR hemoglobin and for protein and protein bound carbohydrate* fractions are given in table V The ESR is higher, the hemoglobin lower and the concentrations of protein bound carbohydrates higher in the clear cell group

Mean differences between pre and post operative levels of these variables are shown in table VI In the granular cell group, only the albumin content is *significantly higher after the operation* In the clear cell group *all* values are significantly altered toward normal levels

Fig 4 demonstrates the correlation — in patients free from metastases — between *elevated ESR and low hemoglobin* in the clear cell group — regression equation $y = -0.03x + 12.9$, sample S D of the regression coefficient $S_b = 0.007$ ($P < 0.001$), and further demonstrates the absence of any such correlation in the granular-cell group ($P > 0.05$)

Discussion

The single symptom in renal carcinoma that has been most discussed is probably fever (for references see (3)) The fever generally has been — and still is — thought to be due to hemorrhage or necrosis in the tumor or to the presence of urinary infection Already in 1911 Israel pointed out that this could not be the explanation He stated that the cause of fever was to be found either in the tumor cells or in the normal cells destroyed by the tumor Bottiger (3) examined 136 cases of renal carcinoma in retrospect and came to similar conclusions Further studies (4, 5) made it probable that the occurrence of fever was connected with a special type of renal carcinoma, the clear cell carcinoma

Fever, elevated ESR and other generalized symptoms may occur when metastases are present, especially when they are widespread (8) But fever may be seen to disappear after nephrectomy even in the presence of metastases (3), and a normal or low ESR does not rule out the presence of metastases (5) To make feasible, as far as possible, an examination of the effect of the tumor *per se, all cases with metastases have been excluded from the following discussion*, regardless of whether the metastases were observed before or at the operation or

appeared only subsequently Of the original 100 cases, there remain for discussion 54, which at the time of follow up investigation 1—3 years after surgical removal of the tumor did not show any signs of metastases There may of course be patients in this group with metastases If, however, metastases cannot be detected more than one year after the operation, it seems reasonable to assume that they were virtually lacking at the time of operation

Three of the 54 patients have been excluded from the discussion of ESR etc because of co existing disease known to cause elevation of ESR, *viz* active renal tuberculosis, polycystic kidney and acute post partem cystopyelitis, and *one* patient because of polycythemia, known to cause a definite decrease of ESR

Based upon the findings that fever and high ESR had been found to be more common in patients with clear-cell than with granular cell carcinoma (5), the cases have been divided according to the predominant cell type The *clear cell group* consists only of patients whose tumors are composed — in all available sections — of more than 50 per cent clear cells the majority of which has a PAS positive cytoplasm

The *granular cell group* consists of the patients with tumors dominated by granular cells always PAS negative in the cytoplasm and together with six cases of clear cell carcinoma likewise with PAS negative cytoplasm

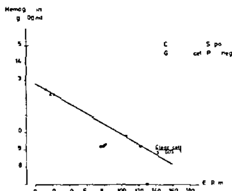
It is sometimes difficult to determine whether a cell or a group of cells should be regarded as clear or granular This difficulty has been encountered in 9 instances but a final classification has been performed in all, and no exclusions have been made for this reason

It should be stressed that most tumors show at least some variation in histology from section to section and that the basis for division has been the *predominance of one cell type* in all available sections This does not necessarily imply that an absolute majority of cells in the tumor are of that special type No way, however has been found to give a better and more definite estimate of the cell population in the tumor

The work of Arner et al (1) suggests that even a few blocks (1—2), when they can be supposed to be cut from representative parts of the tumor make it possible to draw reasonably definite conclusions as to the degree of malignancy This would then probably hold also for division of the tumors into the clear and granular cell type

General symptoms — fatigue, weight loss and fever — seem to be more common in the clear cell than in the granular cell group (table III) This difference is statistically significant The differences between the groups become more distinct if the biochemical findings are analyzed The clear cell group has a higher mean ESR and a lower mean hemoglobin than the granular cell group These differences are highly significant (table V) A significant difference is also found for two serum protein bound carbohydrates (hexosamines and sialic acids) as well as a probable difference for serum protein bound hexose, serum haptoglobin and α_2 globulins No difference was found in albumin and γ-globulin levels

A low albumin content is found as a



F g 4 Correlat on between ESR and hemoglob n in pat ents without metastases w th clear cell ($n = 21$) and granular cell (n 23) tumors respect vely (T me matched values were m ss ng in 6 cases) The regress on l ne and equat on are g ven for the pat ents w h clear cell tumors

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plain the increased ESR as well as the anemia these being the most pronounced alterations in patients with clear cell carcinomas

Such a coating of erythrocytes with proteins has been described in other conditions as during acute myocardial infarction (10) and in diabetes with complications where Ditzel (9) was able to elute α globulin from red blood cells

In this connection the existence of a cancer hormone has been discussed Nakahara and Fukuoka (13) Ohasi and Ono (15) have studied a toxohormone , capable of interfering with liver catalase activity Sylvén and Holmberg (17) have studied another factor, entirely different from that toxohormone They have isolated a polypeptide from interstitial tumor fluid a polypeptide manifesting a very specific reaction with cell surfaces which in certain cases leads to complete inhibition of all membrane activity Their polypeptide has been found to *be absorbed to the cell surface* of human erythrocytes and to influence ESR

Summary

One hundred patients with renal carcinoma have been studied in an attempt to correlate symptoms and findings with the histopathologic picture If patients without apparent metastases were studied general non urologic symptoms including fever seem to be more common in the clear-cell than in the granular cell group

Biochemical alterations increased ESR anemia and also a probable increase in protein bound carbohydrates likewise were more common in the clear cell group

The difference between the activity of clear and granular cell carcinomas was further strengthened through an analysis of pre and post operative differences in the analyzed variables

The possibility is discussed that these alterations are connected with glycoprotein coatings produced by the tumor cells

Acknowledgement

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From the Departments of Medicine (Head P Bechgaard M D) Surgery (Head P Holm Nielsen M D) and Diagnostic Radiology (Head Aa Wagner M D) Aarhus Amtssygehus Aarhus Denmark

Acute Haemorrhage in Peptic Ulcer

A Clinical, Radiographic and Statistical Follow up Study

By

EINAR KRAG

According to Bockus (2), it has been estimated that the ulcer disease in about 25 per cent of all cases will at some time be complicated by manifest bleeding in the form of haematemesis and/or melaena He reckons that this figure is too high being based on analyses of series of hospitalised patients in which the distribution of bleeding and non bleeding ulcers is likely to favour the former category because of differences in the practice of admitting patients of the two categories In this connexion, it may be mentioned that Alsted (1) by means of questionnaires sent to all general practitioners in Denmark in 1940 and 1948 found that haematemesis and/or melaena occurred in 26 and 37 per cent, respectively, of all patients with ulcer disease

Bockus claims that ulcer disease is the cause of haematemesis/melaena in 72—92 per cent of all cases In some of these patients with ulcer bleedings the ulcer cannot be visualised radiologically, but the history is suggestive of ulcer and other causes can be ruled out Holten and Nielsen (6) referred to these patients as X ray negative and found 23 per cent among 397 ulcer bleedings Jones (7) called this group 'acute ulcers and found 32 per cent among 1,794 patients with ulcer bleedings Krarup (9) reported that the X ray negative constituted about 46 per cent among 200 patients, and Wenckert et al (12) found about 16 per cent among 497

The purpose of the study reported here was to throw light on the long term prognosis for patients with ulcer bleedings who had been given medical treatment, and especially to analyse the factors which are of significance in the prognosis

Material and methods

During the period from 1936 to 1945 inclusive a total of 396 ulcer patients who had been treated conservatively were discharged from Aarhus Amtssygehus Of these 107 or 27 % had manifest bleeding evi

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TABLE V Haematemesis/melaena in 1936—1945 Duration of symptoms before first admission

Duration of symptoms (yrs)	X ray positive		X ray negative	
	No	%	No	%
<½	12	21	23	47
½—2	4	7	1	2
2 5	9	15	5	10
>5	33	57	20	41
Total	58	100	49	100

examination As regards the patients who died before the follow up examination, information as to the course of the ulcer disease and the cause of death was obtained by means of death certificates and through the patients own doctors

The hospital records for all patients who had been admitted to hospital for medical or surgical treatment of the ulcer disease during the observation period were reviewed

Clinical cours According to the clinical course of the ulcer disease the patients were divided into three prognostic groups at the follow up examination

Group A Favourable course

Complete freedom of symptoms or only slight dyspepsia not requiring any treatment throughout the observation period

Group B Less favourable course

One re admission and/or one manifest bleeding and/or recurrence of ulcer associated with incapacity for work

Group C Serious course

Two or more re-admissions and/or manifest bleedings or gastric operation or death from ulcer disease

Prognostic significance of individual factors In order to study whether certain factors determined on the first admission in the period 1936—1945 were related to the clinical course of the ulcer disease the three groups just mentioned were compared with regard to the following alternative variables

	Man	Woman
Manifest bleeding before first admission	Yes	No
Duration of symptoms	<	> 2 years
Age	Over	Under 40 years
Hb level	Above	Below 60 %

Statistically the case material was subjected to variance analyses and chi square tests

Results

Clinical course

It appears from table VI that X ray negative bleedings had a significantly milder clinical course than the 'X ray positive bleedings ($p < 0\ 02$) This is in agreement with the results reported by Krarup (9) and Wenckert et al (12)

Recurrent bleeding was seen in about 50 % of the patients during the observation period and was slightly more frequent in X-ray positive than in 'X ray negative' cases, but the difference was not significant (table VII) Wenckert et al found a significant difference with much fewer recurrences of bleeding among the X ray negatives'

Deaths During the observation period death from manifest bleeding occurred

TABLE VI Follow up examination 1963 Clinical course of ulcer disease

	X ray positive						X ray negative					
	♂		♀		Total		♂		♀		Total	
Clinical course	No	%	No	%	No	%	No	%	No	%	No	%
Favourable	9	22	5	33	14	25	5	16	9	56	14	28
Less favourable	6	15	2	13	8	14	12	37	5	31	17	36
Serious	26	63	8	54	34	61	15	47	2	13	17	36
Total followed up	41	100	15	100	56	100	32	100	16	100	48	100
Not followed up	2		0		2		1		0		1	
Total	43		15		58		33		16		49	

TABLE VII Follow up examination 1963 Recurrence of gastric bleeding during observation period

	X ray positive		X ray negative	
	No	%	No	%
Recurrence	32	57	22	46
No recurrence	24	43	26	54
Total followed up	56	100	48	100

TABLE VIII Follow up examination 1963 Frequency of gastric operation during observation period

	X ray positive		X ray negative	
	No	%	No	%
Operation	18	32	6	13
No operation	38	68	42	87
Total followed up	56	100	48	100

in 6 patients (5 6 %) including one in relation to operation Wenckert et al reported a lethality from ulcer bleedings of 4 7 % during observation periods of 9—18 years

Gastric operation During the observation period, operation was performed in 13 % of the "X ray negative" and in 32 % of the "X ray positive" cases (table VIII) The difference is significant ($p < 0.02$)

women However, the difference is significant only in the presence of "X ray negative" findings ($p < 0.01$)

Duration of symptoms Patients who had symptoms for more than 2 years before the first admission to hospital had a poorer prognosis (table XI), but the difference was significant only for those with "X ray positive" findings ($p < 0.05$)

Age at the first admission seems to be only of minor importance Table XII suggests that an age over 40 years, especially in the presence of "X ray negative" findings, is associated with a poor prognosis, but the difference is not significant

The haemoglobin level at the first admission seems to be without any prognostic importance

Discussion

The follow up studies showed that the clinical course is definitely milder for "X ray negative" than for "X ray positive" bleedings In this connexion it may be mentioned that Jones reported that gastroscopy revealed a superficial "acute ulcer" in 37 % of his 'X ray-negative' cases It thus seems as if barium meal examination reveals only coarse, massive changes while superficial ulcers (erosions) will pass unrecognised Nevertheless, barium meal examination *is of great prognostic value* because these bleeding sub radiographic ulcers have a significantly better prognosis than those which are radiographically demonstrable Incidentally, this also applies to non bleeding ulcers (8)

The question as to medical vs surgical treatment of heamatemesis/melaena can not be answered directly on the basis of the present study In the groups of patients who satisfied the criteria for operation set up by Christiansen et al (3), viz a) haematemesis in hospital, b) age over 40 years, c) ulcer unquestionably demonstrated or at least very likely, the lethality observed in the present series was 21 % However, it must be remembered that this series originates from the years 1936—1945, nowadays it may, to some extent, be possible to reduce the mortality by more intensive transfusion therapy In comparison, it may be mentioned that Christiansen et al for the period 1948—1958 found an operative mortality of about 17 % among surgically treated patients who satisfied the above mentioned criteria This observation may well truly signify that there is no definite difference in the *average lethality* from bleeding ulcers, whether medical or surgical treatment is given Similar views were expressed by Holten and Nielsen (6) and Jones (7) In a study of the long term results of treatment of peptic ulcer, in which detailed statistical analyses were performed Krause (10) worked out an "estimation of profit and loss" for surgical and conservative therapy in patients with haematemesis/melaena Krause is in favour of operation after the first recurrence of gastric bleeding but he admits that the indications are not convincing, even in elderly patients

On the basis of the present and previous studies it will presumably be reasonable to conclude that a definitive list of indications for operation cannot

be set up but that each individual case should be assessed on its own merits, in particular, on its response to adequate conservative therapy

Summary

A follow up study of 117 cases of manifest gastric bleeding from the period 1936—1945 is reported Ten deaths occurred during the first admission to hospital giving a lethality of 8 5 per cent The lethality for patients over 50 years was 18 per cent

At the follow up examination in 1963 (17 to 27 years later) information was obtained for 97 per cent of the remaining 107 patients

The patients may be divided into two equally large groups a) X ray positive cases in which an ulcer was demonstrated radiographically during the first hospital stay, and b) "X ray negative cases in which radiography failed to reveal any ulcer lesion

The results of the follow up examination were as follows

1 Of 396 ulcers, 27 per cent were complicated by haematemesis/melaena on the first admission

2 There were twice as many men as women among the patients

3 Patients with X ray negative bleedings on the first admission had had symptoms for a significantly shorter period than those with X ray positive bleedings

4 During the observation period, X ray negative cases had a significantly milder clinical course than the "X ray positive cases

5 The follow up examination showed that the frequency of gastric operation during the observation period was lower among the X ray negative patients

6 Gastric bleeding recurred in about 50 per cent of the patients during the observation period

7 Peptic ulcer subsequently developed in 45 per cent of the "X ray negative cases

8 Manifest bleeding before the first admission resulted in a poorer clinical course during the observation period

9 The prognosis was poorer in men than in women

10 Long duration of symptoms before the first admission resulted in a poorer clinical course during the observation period

The importance of X ray negative findings is discussed The question of medical vs surgical treatment of bleeding ulcers is considered

Acknowledgement

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From Medical Department B (Heads Kai Schmith and Svend Norregård), Frederiksberg Hospital, Copenhagen Denmark

Angiotensin Infusion Test in Arterial Hypertension

By

B Weeke and A R Krogsgaard

The role of renin angiotensin in human arterial hypertension remains unelucidated However, there is some evidence of a relationship in renovascular hypertension, in animal experiments (3, 4 12) as well as in man (2 5 6 12 13)

Analyses for renin and angiotensin are as yet restricted to a few laboratories Therefore it was of considerable interest when Kaplan and Silah (8, 9) published their studies on the infusion test with angiotensin Using a standardized infusion with measurement of the pressor effect these authors could differentiate various forms of hypertension The curable cases of renovascular hypertension showed less sensitivity than normotensives to angiotensin while patients with essential hypertension were hyper reactive The method was found to have somewhat limited applicability, patients with malignant and accelerated hypertension being less sensitive even in the absence of renal artery stenosis Similarly secondary hyperaldosteronism caused by restricted salt intake diuretic therapy, cirrhosis or nephrosis entailed a reduced pressor effect of angiotensin

The simplicity of the method, and the consequent possibility of its routine use in any hospital called for further trials

Technique and material

Kaplan and Silah (8 9) in the initial investigations used increasing doses of angiotensin intravenously until the diastolic blood pressure rose by 20 mm Hg They found that patients with renovascular hypertension required more than 6 5 mμ/kg/min while nearly all patients with hypertension of other origin reacted to less than 5 mμ/kg/min On this basis they reported that a single dose of 4 mμ/kg/min administered intravenously over 5 min was applicable as a screening test

In our studies therefore we used this dose initially If it did not entail an increase of at least 20 mm Hg diastolic we administered 6 5 and in some cases also 8 mμ/kg/min

The investigation was done on in patients in all cases except for two who were out patients After an intravenous drip of physiological saline had been established with a minimal drop rate the blood pressure was checked by auscultation until stable There

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TABLE I The dose of angiotensin required to increase the diastolic blood pressure by 20 mm Hg in 50 patients with normal blood pressure or different types of hypertension

Clinical groups	Pressor dose of angiotensin (mμ/kg/min)				No of pat
	Diastolic BP increase ≥ 20 mmHg				
	4	6 5	8	>8	
Normotensives	2	7	1		10
Essential benign hypertension	6	10	5	2	23
Accelerated hypertension	1				1
Malignant hypertension		1			1
Chronic pyelonephritis with hypertension		1			1
Polycytic kidney with hypertension				1	1
Unilateral hydronephrosis with hypertension	1	1			2
Essential hypertension with ab normal isotope nephrography	2	3	2		7
Renal artery stenosis with hypertension		3		1	4

after the above mentioned dose of angio tensin in a solution containing 1 μg per 10 ml was injected into the tubing without the patient being aware of it The blood pressure was measured every half to whole minute during the 5 minute infusion

The composition and size of the material is shown in table I It comprises 50 persons including a normotensive control group of 10 with no signs of cardiovascular or renal disease The 40 patients had hypertension of diverse genesis In addition to the history taking and a clinical examination all the patients had urine analysis for albumin microscopic examination of the urine determination of serum creatinine serum sodium potassium chloride and total CO ophthalmoscopy intravenous rapid sequence pyelography and isotope nephrography with [131]I hippuran (however, in one of the patients the last mentioned investigation was not performed) Eight patients more over, had renal angiography and 4 the Howard test

The material was grouped on the basis of these investigations

The patients were on an ordinary hospital diet Diuretics were being administered to 7 patients one patient was also receiving methyldopa and one guanethidine

Results

The results are apparent from the table, which shows the distribution of the patients according to the dose of angio tensin required to raise the diastolic pressure by at least 20 mm Hg

A comparison of the normotensive subjects with the patients having essen tial benign hypertension shows that the latter did not have definitely greater sensitivity to angiotensin such as was expected from the literature Seven out of the 23 even gave a sluggish response

to angiotensin In 4 of these 7 patients renal angiography did not show signs of renal artery stenosis, 2 were not subjected to angiography In the seventh patient autopsy later showed normal renal arteries but an aneurysm in the abdominal aorta, so that it is difficult to rule out renal ischaemia in this case

Unfortunately, we have only one patient with malignant and one with accelerated hypertension Nevertheless, these two patients are worth mentioning because — contrary to Kaplan and Silah s experience — they did not require particularly high doses of angiotensin

The groups of various renal disorders are too small to allow any conclusions The reason why the patient with polycystic kidney gave only a slight response to angiotensin is perhaps intrarenal ischaemia

Most interest attaches to the 4 patients with radiological evidence of unilateral renal artery stenosis As is apparent only one of these patients responded as might be expected according to Kaplan and Silah Regrettably, a Howard test could not be carried out on this patient as she had hydronephrosis and defective excretion on the contralateral side Among the other 3 patients, we should like to emphasize in particular a 37 year old woman with arteriographic evidence of fibromuscular hyperplasia of the right renal artery and a corresponding quite obvious renal ischaemia in the Howard test In spite of these conclusive findings the patient responded normally to the infusion of angiotensin In the last 2 patients with renal artery stenosis (one of them was a patient in the Medical Department of Diakonissestiftelsen, Copenhagen) we cannot state anything definite concerning renal ischaemia, as the Howard test was a technical failure in one and was not carried out, for various reasons in the other

According to Kaplan and Silah administration of diuretics reduces the sensitivity to angiotensin but we could not confirm this finding Out of the 7 patient who were being treated with thiazide at the time of the angiotensin infusion, 2 responded to 4 mμ/kg/min and the other to 5—6 5 mμ/kg/min

Discussion

Our series of 50 persons is inadequate for elucidating the value of the angiotensin infusion test in every detail However, we feel in a position to draw certain conclusions predominantly on the basis of the discrepancies between our results and Kaplan and Silah s

Angiotensin infusion, in this simple form, does not permit a definite recognition of renovascular hypertension in all cases as one of our patients with obvious renal ischaemia did not show a reduced sensitivity to angiotensin The same finding was made by Morgan (11) in 2 patients with renal artery stenosis whose blood pressure returned to normal after surgery A reduced sensitivity to angiotensin was found in our series in about one third of the patients with essential hypertension A similar observation was made by Wax (14) in 21 out of 46 patients Among other findings of ours which do not accord with Kaplan and Silah s it is worth mentioning the normal response

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From the Medical Department (Head Erik Ask Upmark M D) and the Department of Clinical Physiology (Head Gunnar Strom M D) University of Uppsala Uppsala, Sweden

Effects of Hypercapnoea and Hyperoxia on Pulmonary Circulation in Patients with Bronchial Asthma

By

LARS IRNELL and LARS NORDGREN

In a previous study (9) we found indications that in patients with bronchial asthma pulmonary vasoconstriction of a not insignificant degree was present — despite the absence of pulmonary hypertension — even during a so called attack-free interval It was noted that during infusion of acetylcholine into the pulmonary artery there was such a large average reduction of the arterial oxygen saturation that it corresponded to a shunt ' past non ventilated lung regions of 20—25 % of the cardiac output

Several authors have previously studied in animals and in healthy subjects the effect of acute hypercapnoea on the pulmonary circulation (2, 3, 5, 10 11, 13) In a few investigations the subjects have consisted of patients with chronic pulmonary disease (7, 12), mainly with pulmonary emphysema and respiratory insufficiency, not seldom complicated by cor pulmonale We have found no published reports of investigations in which the effect of a temporary increase of the carbon dioxide in the inspired air on the pressure and flow conditions in the pulmonary circulation has been studied in patients with uncomplicated bronchial asthma This experiment therefore was performed in the present study

Nor does any study appear to have been made on the haemodynamic effects of hyperoxia in a similar series of patients It has been shown (3) in patients with different types of chronic pulmonary disease — mainly chronic bronchitis — that during oxygen breathing there is a tendency to reduction of the pul[illegible] frequency, cardiac output and the mea[illegible] pressure in the pulmonary artery

As a complement to our investi[illegible] on the effects of hypercapnoea, [illegible] report and discuss below some [illegible] dynamic data from a partial [illegible] which asthma patients inhaled [illegible] of equal parts of air and pure [illegible]

The definition of bronc[illegible] followed the recommenda[illegible]

Submitted for publication February 23 1966

TABLE I Mean, highest and lowest values for certain case history factors and tests measuring lung

	Age	Sickness points total	Age at onset	Average no of sickness points during the last 5 years	Duration (year)	BSA (m^2)	VC (%)
Mean	45	1 779	23	47	21	1 72	91 2
Highest	59	7 781	46	142	50	2 20	111 4
Lowest	30	7	2	0	1	1 44	64 5

TABLE II Mean highest and lowest values for certain function variables determined at right heart

	O_2 saturation (%)							
	Br A		PA		AV diff (ml/l)		Oxygen uptake (ml/l)	
	C	T	C	T	C	T	C	T
Mean	97 1	99 0	72 3	75 1	44 7	42 9	216	215
Highest	99 7	100 0	81 4	82 1	59 7	56 8	288	294
Lowest	93 1	95 3	60 7	61 7	29 7	28 1	171	157

	Pressure Br A (mm Hg)							
	S		D		M		Pa_{CO} (mm Hg)	
	C	T	C	T	C	T	C	T
Mean	142	156	77	84	103	115	37 5	42 0
Highest	190	215	110	120	140	165	49 0	51 0
Lowest	110	110	55	60	80	85	30 5	27 5

by the American Thoracic Society (1) No patients with chronic bronchitis were included in this study

Material

The hypercapnoea series comprised 40 patients and the hyperoxia series 58 cases All of these patients were included in the material of bronchial asthma patients whose selection has been described in detail by Irnell (8) The patients who underwent the present investigation were chosen at random from this latter material Accordingly both the data concerning the degree of severity of the disease and the values for the different cardio-pulmonary functions, for these two series of patients, did not differ essentially

function and physical work capacity for the 40 patients constituting the hypercapnoea series

$FEV_{1.0}$ (%)	FEV (%)	MVV_{40} (%)	MVV_F (%)	FRC/ TLC (%)	RV/ TLC (%)	Eqv time (min)	PWCmax abs (kpm/ min)	PWCmax (%)
75 3	81 5	65 6	65 1	112 8	137 6	4	618	73 7
116 3	113 6	102 8	119 0	150 0	196 0	8	1 500	125 3
39 1	46 2	33 9	33 8	84 4	88 8	2	200	35 8

catheterization before (C) and during (T) inspiration of 5% CO_2 in air in 40 patients

Cardiac output (l/min)		Stroke volume (ml)		Heart rate (beats/min)		Pressure PA (mm Hg)					
						S		D		M	
C	T	C	T	C	T	C	T	C	T	C	T
5 0	5 0	67	65	76	80	19	24	7	9	12	15
8 0	8 1	124	117	95	114	30	45	14	23	20	32
3 1	3 3	41	39	56	57	12	15	2	4	5	9

Pa_{CO_2} (mm Hg)		Ventilation (l/min) BTPS		pH arterial		HCO (mEq/l)	
C	T	C	T	C	T	C	T
95	95	6 90	20 91	7 39	7 35	22 1	21 8
108	139	11 17	41 38	7 51	7 46	26 0	26 0
86	85	4 83	10 50	7 32	7 24	17 0	17 0

from those reported in the previous work (8) With the method (8) used for evaluating the degree of severity of the disease from the case history it was found that in the hyper capnoea series 13 patients had had very severe asthma 13 severe asthma and 14 moderately severe asthma. The corresponding figures for the hyperoxia series were 18 21 and 19 respectively The ages at onset of the asthma and the duration of the disease were 23 and 21 years respectively for the hypercapnoea series and 26 and 20 years for the hyperoxia series

Methods

Right heart catheterization was performed with the usual technique The cardiac output

TABLE III Statistical data for differences between haemodynamic values during CO_2 breathing and during air breathing determined at heart catheterization The degrees of significance are denoted by asterisks

		Pressure PA		Pressure Br A	
	O_2 sat Br A	S	M	S	M
n	39	38	38	39	37
Mean difference $\bar{d}$	1 77	5 18	3 34	15 21	13 51
S D of difference	1 71	5 54	4 14	21 44	18 14
S E of mean diff	0 27	0 90	0 67	3 43	2 98
$t=\bar{d}/S E$	6 46***	5 77***	4 97***	4 43***	4 53***
Neg difference (no of pat)	5	5	5	5	1

was determined according to the direct Fick principle

Expiratory air was collected with the patient at rest starting 3 minutes after the insertion of the mouthpiece and continuing for 10 minutes Pressure recordings were made and blood samples taken during the fifth to eighth minute of gas collection

For the hyperoxia test the patient breathed a mixture of about 50 % oxygen (mean 49 27 %, lowest 47 25 %, highest 52 28 %) in nitrogen for 18 minutes Expired air was collected during the 8th to 18th minute Pressures were recorded and blood samples taken during the 13th to 17th minute

For the hypercapnoea test the patient breathed a mixture of about 5 % carbon dioxide (mean 5 39 %, lowest 5 11 %, highest 5 88 %) in air After an initial period of 5 minutes pressures were recorded blood *samples taken and expired air collected* during a period of as a rule 5 minutes (range 4—8 minutes)

For more details of the methods for right heart catheterization and analysis of blood samples and circulatory and respiratory function, see Irnell (8)

Results

Hypercapnoea Table I shows the composition of the material with regard to certain relevant background data, e g the assessed degree of severity of the disease and data representing the ventilation capacity, lung volumes and physical work capacity As may be seen, the maximal voluntary ventilation capacity was on the average 65 % of the estimated normal value, the ratio of the residual volume to the total lung capacity was 138 % of the estimated normal value and and the physical work capacity in submaximal testing was 74 % of the estimated normal value

Table II shows the highest, lowest and mean values for a number of function variables both under ordinary resting conditions and during hypercapnoea This table gives the impression that both the arterial oxygen saturation and the pressure in the pulmonary and brachial arteries increase during CO_2 breathing In order to test more stringently whether the above mentioned functions underwent a true change during the hypercapnoea, table III was prepared This is based on individual differences between the control and test values The

TABLE IV Coefficients of correlation illustrating the relationships between certain lung function values and differences for certain haemodynamic functions before and during CO_2 breathing The degrees of significance are denoted by asterisks

	VC (%)	$FEV_{1.0}$ (%)	FEV (%)	MVV_{40} (%)	MVV_F (%)	FRC/TLC (%)	RV/TLC (%)	PWC max
O_2 sat Br A	−0.03	−0.07	0.00	0.00	0.10	0.11	−0.02	0.08
Pressure PA syst	−0.09	−0.08	0.05	−0.25	−0.24	0.24	0.19	−0.29
Pressure PA mean	−0.13	−0.14	−0.03	−0.33*	−0.34*	0.25	0.14	−0.26
Pressure Br A syst	−0.09	−0.04	0.02	−0.03	0.04	0.16	−0.11	0.00
Pressure Br A mean	0.05	0.02	0.09	0.05	0.07	0.29	0.08	0.09

table only shows those function variables from table II which were found to be of especial interest

In table III clearly significant (***) effects of the CO_2 breathing are evident, i e the mean figures have risen It may be seen however, that occasional patients show negative differences The majority of these negative differences are so small that they could be explained by the random error of the method One patient was affected by a vasovagal fall in arterial blood pressure It is this patient who exhibits a negative difference for function no 5 i e the mean pressure in the brachial artery

It was possible in this series of patients to study whether the individual differences caused by hypercapnoea were correlated to the patient s lung function Negative coefficients of correlation would not be unexpected since it could probably be assumed that patients with greater lung function reduction would react more strongly to CO_2 administration than those in whom the lung function was less abnormal As may be seen in table IV, a number of such correlations were found, in particular the relationship between, on the one hand arterial oxygen saturation and pressure in the pulmonary artery and on the other hand ventilation capacity Only the correlations between, on the one hand, MVV_{40} and MVV_F and on the other hand $\bar{P}_{PA}$ were however significantly (*) different from 0

Table V shows the relationship between on the one hand a number of case history factors such as age sickness points (total number since onset of disease) sickness points for the 5 year period immediately preceding the investigation (8) age at onset and duration of the disease, and on the other hand the functions given in table III which were studied in connection with heart catheterization Positive correlations were to be expected between these functions and the age of the patient number of

TABLE V Coefficients of correlation illustrating the relationships between certain case history data and haemodynamic functions before and during CO_2 breathing

	Age	Sickness points	Average no of sick ness points during the last 5 years	Age at onset	Duration of disease
O_2 sat Br A	0 17	0 15	0 27	0 05	0 16
Pressure PA syst	0 31	0 27	−0 05	0 13	0 11
Pressure PA mean	0 32	0 28	−0 03	0 14	0 15
Pressure Br A syst	0 28	0 07	0 20	0 21	0 05
Pressure Br A mean	0 02	−0 02	0 07	−0 01	0 06

sickness points and the duration of the disease Such correlations were in fact found, but the material was not large enough for statistically significant rela tionships to be demonstrated The results shown in the table are, however, not contrary to the expectations

Hyperoxia A series of 58 patients were studied during inspiration of 50 % oxygen in nitrogen Owing to methodolog ical uncertainty in the cardiac minute-volume determination according to the Fick principle under the present experi mental conditions, the results obtained are not reported in detail, but the haemodynamic values obtained are sum marized below Also omitted is a description of the composition of the material as regards certain background data, such as the assessed degree of severity of the disease and the values for ventilation capacity, lung volumes and physical work capacity These data — as also the haemodynamic data under resting conditions — closely accord with these reported earlier (8)

During hyperoxic breathing the value for oxygen uptake was found to rise to an average of 267 ml/min from an average of 217 ml/min under ordinary resting conditions Such a large increase of metabolic oxygen uptake would be unexpected, and an unsteady state concerning oxygen distribution in the lung may have contributed The cor responding values for the cardiac output were calculated as 4 9 and 5 9 l/min, respectively The mean pressure in the pulmonary artery under ordinary resting conditions was on the average 12 mm Hg, as compared with 11 mm Hg during hyperoxic breathing The oxygen saturation in the brachial artery was on the average 96 4 % under resting conditions and 99 6 % during hyperoxic breathing

Discussion

In this series of patients with isolated or 'uncomplicated" bronchial asthma, normal pressures and flows in the pul monary circulation were recorded at rest Lung function data showed signs of at least a moderate degree of hyper inflation in the lungs (measured as RV/TLC) The gas distribution in the lungs was not measured directly, but it

seems reasonable to conclude from the data obtained that this was to a certain extent disturbed The fact that the arterial oxygen saturation was not on the average significantly decreased should mean that poorly ventilated lung regions also exhibited reduced perfusion The restriction in perfusional area was not however, so great as to be reflected in a raised pressue in the pulmonary circula tion The effects of acetylcholine infu sion and anoxic anoxia on the pulmonary circulation in our patients were described previously (8, 9) It was reported then that during anoxic anoxia (11 % $F_{I_{O_2}}$) resulting in an avergae arterial oxygen saturation of 76 %, there was a signifi cant pressure increase in the pulmonary circulation without any observed change in cardiac output During acetylcholine infusion into the pulmonary artery under resting conditions, a significant reduction of the arterial oxygen satura tion was found with no change in cardiac output These results suggested that anoxia produced a decrease of the per fusion and that acetylcholine increased the perfusion to poorly ventilated lung regions In the investigations reported in the present paper an increase in oxygen uptake, and in cardiac output, may have occurred during hyperoxia (50 % $F_{I_{O_2}}$) while the arterial pressure in the pulmonary circulation was not materally changed compared with the resting values The values for metabol ic oxygen uptake are, however, uncertain, and it cannot be concluded from these findings that during hyperoxia, with the lessening of anoxia in slowly ventilated lung regions, there is increased pulmonary perfusion

In the interpretation of the results of hypercapnoeic breathing the investiga tional conditions should be taken into consideration When CO_2 is given as a constant fraction of the inspired gas the quantity of CO_2 administered is depend ent on the minute ventilation, and the alveolar pCO_2 cannot be decreased by hyperventilation below the tension of inspired gas (6) In our investigations with hypercapnoea we could not rely on definite "steady state" conditions for the minute ventilation This consti tuted a source of error for the deter mination of the cardiac output We found, however, during hypercapnoea, a significant pressure rise in the pulmo- nary circulation and also a tendency to an increase of the arterial oxygen satura tion with no signs of change in oxygen uptake, cardiac minute volume or arterial pO_2 It seems probable from these results that lung regions with lowered ventilation and perfusion did reduce their perfusion further during hypercapnoea Our results allow no definite conclusions to be drawn as to where the respiratory factors affect the pulmonary vascular bed

Summary

The effects of hypercapnoeic or hyper- oxic breathing on the pulmonary circula tion were studied during symptom free intervals in patients with uncomplicated bronchial asthma, who under resting conditions were normotensive in the pulmonary circulation

During inspiration of 5 % CO_2 in air the arterial oxygen saturation rose slightly, and the pressure in the pul

monary and brachial arteries rose significantly The increase in mean pressure in the pulmonary artery was found to be related to the reduction of the maximal voluntary ventilation capacity Otherwise no statistically significant relationships were found between the changes in certain haemodynamic functions on the one hand and factors constituting a measure of the degree of severity of the disease, ventilation capacity, lung volumes and physical work capacity, on the other No relevant changes were observed during inspiration of 50 % oxygen in nitrogen

Statistical adviser Gunnar Eklund, Ph D

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From the Renal Clinic and the Central Laboratory St Erik s Hospital, Stockholm Sweden

The Effect of Thiazides, Chlorthalidone and Furosemide on Muscle Electrolytes and Muscle Glycogen in Normal Subjects

By

Jonas Bergström and Eric Hultman

Diuretics of the thiazide type, including chlorthalidone, promote the excretion of water, sodium and chloride during the initial phase of treatment The excretion of potassium also shows an increase though generally to a smaller degree The result of treatment with thiazides is a condition involving decrease of total body water, extracellular fluid and plasma volume A hypochloremic alkalosis with hypokalemia is found in the plasma

Furosemide is a new, very potent sulphonamide diuretic The effect on the sodium and chloride excretion equals or exceeds that of the thiazide diuretics (16, 21 32) Furosemide has also been reported to increase the potassium excretion (28, 32) and give rise to hypokalemia (3, 16)

There is still a difference of opinion whether hypokalemia in thiazide treatment is a sign of actual potassium deficiency or due to a redistribution of the potassium between the extracellular fluid and the cells Balance studies by Sandoe and Olesen (23) disclosed a negative potassium balance in normal subjects and patients given a diet with a constant electrolyte content A 5 to 10 per cent decrease of total exchangeable potassium was found by Hollander et al (10) after 4—8 weeks of chlorothiazide treatment Kaul et al (15) found a 7 7 per cent decrease of total body potassium in normal subjects (K^{40} measured with whole body counter) after treatment with chlorthalidone Gifford et al (8) observed a fall in total exchangeable potassium during the first two weeks of thiazide treatment of patients with hypertension, but thereafter the values tended to return towards the values preceding the treatment Talso and Carballo (31) determined the total exchangeable potassium, before and after four weeks of thiazide treatment of hypertensive patients but could not discern any significant effect

Rooth and Fürst (22) studied the relationship between serum potassium and base excess in patients with hypertension and cardiac insufficiency treated with polythiazide or chlorthalidone

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They assumed the hypokalemia to be attributable to the extracellular alkalosis

In a preliminary investigation in this laboratory a series of patients with hypertension was studied before and after one week of chlorthalidone treatment A statistically significant decrease of muscle potassium, and a probably significant increase of intracellular sodium occurred (2)

Attention has additionally been paid in the past few years to the effect of diuretics of the thiazide type on carbohydrate metabolism These diuretics are known to be capable of precipitating diabetes mellitus and aggravating pre-existing diabetes (9, 25)

It emerged from our preliminary investigations that some of these diuretics cause a decrease of muscle glycogen in normal subjects Given that muscle glycogen synthesis depends on an unimpaired carbohydrate metabolism (4), a decrease of muscle glycogen after treatment with diuretics might be a manifestation of the diabetogenic action

The purpose of the present investigation was to study in normal subjects

a) the electrolyte changes in muscle tissue after administration of diuretics,

b) the effect of different diuretics on the glycogen content in muscle, i e to ascertain whether the preliminary results obtained earlier could be verified

The effect of the following four drugs was examined hydrochlorothiazide (Dichlotride®, Merck, Sharp & Dohme) polythiazide (Renese®, Pfizer) chlorthalidone (Hygrotone®, Geigy), and furosemide (Lasix®, Hoechst)

Material and methods

Volunteer normal subjects aged 18—45, were examined by means of needle biopsies taken from *musculus quadriceps femoris* before and after seven days treatment with one of the diuretics mentioned below Coincident with the biopsies arterial blood samples were taken Plasma electrolytes plasma protein and blood glucose were determined The body weight was recorded During the experimental period the subjects were on a free diet without any restrictions in water or electrolyte intake They carried on their usual daily work Some of them experienced side effects in the form of nausea and marked fatigue during the first 2 or 3 days, after which the symptoms diminished or disappeared All four drugs revealed similar side effects

In 3 subjects the experiment had to be interrupted owing to nausea and vomiting (2 after chlorthalidone, 1 after polythiazide) These cases were excluded The distribution of the remaining material and the dosages were as follows

1 a *Hydrochlorothiazide* Ten subjects (5 men 5 women), 100 mg (2 doses of 50 mg each) daily No electrolyte studies were performed in these 10 subjects

1 b *Hydrochlorothiazide* Ten subjects (5 men 5 women), 150 mg (3 doses of 50 mg each) daily Electrolyte studies were performed in all the subjects

2 *Polythiazide* Eighten subjects (9 men 9 women), 4 mg (2 doses of 2 mg each) daily Electrolyte studies were performed in 8 subjects (2 men 6 women)

3 *Chlorthalidone* Twelve subjects (8 men 4 women) 200 mg (2 doses of 100 mg each) daily Electrolyte studies were performed in 8 subjects (4 men, 4 women)

4 *Furosemide* Twenty two subjects (8 men 14 women) 100 mg (4 doses of 25 mg each) daily Electrolyte studies were performed in 10 subjects (6 men 4 women)

On the first day of examination after the biopsy had been made the subjects immediately began to take in the diuretic continuing for 7 days On the morning of the 8th

day they were given a las single dose of the diuretic, and then re-examined by biopsy and blood sampling The biopsies before and after treatment were performed at the same time of the day The subjects had been fasting since 8 p m the previous evening

Needle biopsy specimens were obtained from the lateral portion of musculus quadriceps femoris after local anesthesia of the skin (1) The biopsy specimens were taken alternately from the two legs By repeated sampling at least four specimens (5—10 mg each) were obtained at each biopsy two for glycogen and two for water and electrolyte assays

The specimens for the electrolyte determination were analyzed ad modum Bergstrom (1) for water sodium chloride potassium and phosphorus with use of neutron activation analysis for the electrolyte determinations The neutron irradiations were carried out in reactor R 1, AB Atomenergi Stockholm Specimens from one single subject taken before and after the administration of the diuretic were always irradiated together as close as possible to reduce the influence of neutron flux variations

Water and electrolyte content in muscle tissue were referred to fat free solids (FFS) The chloride space (H_2O_{Cl}) and excess sodium i e sodium not confined to the chloride space were calculated as earlier described (1)

The potassium content was calculated on the basis both of fat free solids and of total phosphorus The latter has certain advantages as a basis of reference owing to *a* the phosphorus being almost exclusively localized intracellularly and *b* intracellular phosphates representing the majority of the intracellular anions

The muscle specimens for glycogen determination were homogenized in water, and protein was precipitated with trichloracetic acid within 10 min after the removal of the biopsy material The supernatant was hydrolyzed with sulphuric acid and the glucose formed was determined by the orthotoluidine method (12) A detailed description of this method will be published shortly (13)

Plasma electrolytes and protein were analysed according to current methods (1) pH, standard bicarbonate and P_{CO_2} were determined in whole blood (26) Blood glucose was determined by the orthotoluidine method ad modum Hultman (12)

Results

1 *Body weight and plasma protein* (tables I A, II A, III A, IV A)

In all the series a decrease in body weight averaging about 2 kg was recorded An increase of the plasma protein concentration was also obtained This change was least marked with hydrochlorothiazide (+ 6 6 g/l), and most marked with furosemide (+ 12 g/l, corresponding to a 14 per cent reduction of the plasma volume)

2 *Plasma electrolytes and acid base equilibrium* (tables I A, II A, III A, IV A)

In all the series a hypochloremic alkalosis was obtained The biggest increase in the standard bicarbonate level was recorded in the subjects treated with chlorthalidone and polythiazide (4 0 and 3 9 mEq/l resp) Smaller changes were noted with hydrochlorothiazide and furosemide (+ 3 2 mEq/l) All the series showed an increase of Pco , but this was not significant with polythiazide The rise in P_{CO_2} suggests a respiratory compensation of the metabolic alkalosis The pH variations were relatively small (the biggest increase was 0 05 unit after polythiazide administration) The plasma potassium concentration fell significantly in all the series This fall equalled 1 1 mEq/l after hydrochlorothiazide polythiazide and

TABLE I Results in 10 normal subjects Hydrochlorothiazide 150 mg daily during 1 week Mean values before and after drug administration and statistical data

	Mean value				
	Before $\overline{m_1}$	After $\overline{m_2}$	Difference $\overline{m_2}-\overline{m_1}$	t	Significance
A Body weight and plasma values					
Body weight (kg)	62 9	61 0	−1 9	7 16	***
Plasma protein (g/l)	68 3	74 9	6 6	8.82	***
Blood pH	7 39	7 42	0 03	5 80	***
P_{CO_2} (mm Hg)	40 3	44 6	4 3	3 81	**
Standard bicarb (mEq/l)	23 5	27 1	3 6	5 34	***
[Cl]⁻ (mEq/l)	106 3	96 1	−10 2	9 70	***
[Na] (mEq/l)	140 4	139 4	− 1 0	1 43	—
[K] (mEq/l)	3 81	2 73	− 1 08	17 1	***
Phosphate (mg/100 ml)	3 19	2 59	− 0 60	3 54	**
B Muscle values per 100 g fat free solids					
Total H_2O (ml)	346	332	−14	2 24	—
H_2O_{Cl} (ml)	72 9	51 3	−22 0	4 62	**
(Total $H_2O-H_2O_{Cl}$) (ml)	273	282	9	1 52	—
Cl^- (mEq)	8 6	5 5	− 3 1	5 94	***
Na^+ (mEq)	11 1	9 5	− 1 6	2 59	*
Na_x^+ (mEq)[1]	0 8	2 4	1 6	5 18	***
K^+ (mEq)	44 8	42 1	− 2 7	3 12	*
Phosphate (mM)	29 2	29 4	0 2	0 42	—
K/P (mEq/mM)	1 55	1 45	− 0 10	6 15	***

[1] Na_x = sodium not confined to the chloride space

$t = \frac{\text{mean diff}}{e_{diff}}$ where e_{diff} = standard error of the mean difference

* = P < 0 05
** = P < 0 01
*** = P < 0 001

chlorthalidone administration After furosemide the decrease was 0 64 mEq/l None of these drugs produced any significant change in plasma sodium concentration Plasma chloride decreased with all four drugs

A certain decrease of phosphate in plasma was obtained in all the series, being significant after administration of hydrochlorothiazide, polythiazide and chlorthalidone

3 *Water and electrolytes in muscle tissue* (tables I B, II B, III B)

a) The effect of hydrochlorothiazide, polythiazide and chlorthalidone

TABLE II Results in 8 normal subjects Polythiazide 4 mg daily during 1 week. Mean values before and after drug administration and statistical data

	Mean value				
	Before $\overline{m_1}$	After $\overline{m_2}$	Difference $\overline{m_2}-\overline{m_1}$	t	Significance
A Body weight and plasma values					
Body weight (kg)	61 6	59 5	− 2 1	6 08	***
Plasma protein (g/l)	68 4	74 3	7 8	5 44	***
Blood pH	7 38	7 43	0 05	4 55	**
P_{CO} (mm Hg)	43 0	46 0	3 0	0 92	—
Standard bicarb (mEq/l)	23 8	27 7	3 9	3 53	**
[Cl] (mEq/l)	105 8	94 1	−11 7	10 49	***
$[Na]^+$ (mEq/l)	140 6	139 3	− 1 3	2 31	—
[K] (mEq/l)	3 81	2 70	− 1 11	14 07	***
Phosphate (mg/100 ml)	3 23	2 66	− 0 59	3 13	*
B Muscle values per 100 g fat free solids					
Total H_2O (ml)	340	328	−12	2 89	*
H_2O_{Cl} (ml)	69 1	54 6	−14 5	2 60	*
(Total $H_2O-H_2O_{Cl}$) (ml)	270	274	4	0 29	—
Cl (mEq)	8 1	5 7	− 2 4	3 56	**
Na (mEq)	10 2	9 4	− 0 8	1 61	—
Na_x (mEq)	0 6	1 7	1 2	2 76	*
K (mEq)	44 2	41 5	− 2 7	3 14	*
Phosphate (mM)	29 4	29 1	− 0 3	0 53	—
K/P (mEq/mM)	1 51	1 43	− 0 08	2 24	—

Symbols as in table I

Total water decreased slightly The decrease was significant ($p < 0\ 05$) after polythiazide and chlorthalidone administration

The muscle chloride content as well as the chloride space of the muscle tissue decreased significantly after the three drugs

The total sodium content fell slightly but this was not significant in any single series

Excess sodium, i e sodium outside the chloride space, was found to increase with hydrochlorothiazide, polythiazide, and chlorthalidone

A decrease of the muscle potassium content was found in all three series The decrease was of the same magnitude (2 7—2 9 mEq/100 g FFS, $p < 0\ 05$) *Also the potassium/phosphorus ratio* was lowered, significantly so in the cases treated with hydrochlorothiazide ($p < 0\ 001$) and chlorthalidone ($p < 0\ 05$)

TABLE III Results in 8 normal subjects Chlorthalidone 200 mg daily during 1 week Mean values before and after drug administration and statistical data

	Mean value				
	Before $\overline{m}$	After $\overline{m_2}$	Difference $\overline{m_2}-\overline{m_1}$	t	Significance
A Body weight and plasma values					
Body weight (kg)	62 3	60 5	− 1 8	7 48	***
Plasma protein (g/l)	67 6	76 0	8 4	7 95	***
Blood pH	7 39	7 42	0 03	3 67	**
P_{CO_2} (mm Hg)	40 4	45 6	5 2	2 76	*
Standard bicarb (mEq/l)	23 2	26 8	3 6	4 79	**
[Cl] (mEq/l]	105 3	96 3	− 9 0	4 99	**
[Na] (mEq/l)	140 0	139 0	− 1 0	0 46	—
[K] (mEq/l)	3 68	2 53	− 1 15	6 34	***
Phosphate (mg/100 ml)	*3 48*	2 99	*− 0 49*	*3 17*	*
B Muscle values per 100 g fat free solids					
Total H_2O (ml)	347	337	−10	2 41	*
H_2O_{Cl} (ml)	79 4	50 3	−29 1	4 27	**
(Total H_2O—H_2O_{Cl}) (ml)	268	286	18	2 35	—
Cl (mEq)	9 3	5 4	− 3 9	4 45	**
Na^+ (mEq)	12 7	10 4	− 2 3	1 93	—
Na_x (mEq)	1 3	3 4	2 1	4 22	**
K (mEq)	43 8	40 9	− 2 9	3 34	*
Phosphate (mM)	28 8	28 9	0 1	0 97	—
K/P (mEq/mM)	1 51	1 40	− 0 11	3 34	*

Symbols as in table I

No significant changes were obtained in total muscle phosphorus

The changes produced by hydrochlorothiazide, polythiazide, and chlorthalidone showed, on the whole, the same tendency and order of magnitude, denoting that the effects of these diuretics were comparable There was, accordingly, justification for dealing with them statistically as one single material to ensure greater accuracy in establishing the typical effects on muscle electrolytes The statistical analysis of the combined material is presented in table V

The results may be summarized as follows After one week's treatment with a diuretic of the thiazide type, a loss in total muscle water was observed amounting to 12 ml/100 g FFS, or 3 5 per cent of the basal value The muscle chloride content decreased markedly as did also the chloride space This latter decrease amounted to 22 ml, i e

TABLE IV Results in 10 normal subjects Furosemide 100 mg daily during 1 week Mean values before and after drug administration and statistical data

	Mean value				
	Before $\overline{m}$	After $\overline{m}$	Difference $\overline{m}-\overline{m}_1$	t	Significance
A Body weight and plasma values					
Body weight (kg)	71 1	68 9	− 2 2	7 51	***
Plasma protein (g/l)	69	81	12	11 02	***
Blood pH	7 40	7 42	0 02	4 04	**
P_{CO} (mm Hg)	40 5	44 8	4 3	2 43	*
Standard bicarb (mEq/l)	23 7	26 9	3 2	6 21	***
Cl (mEq/l)	108 1	100	− 8 1	9 54	***
Na (mEq/l)	139 0	139 4	0 4	0 65	—
K (mEq/l)	3 78	3 14	− 0 64	11 02	***
Phosphate (mg/100 ml)	3 07	2 87	− 0 20	1 64	—
B Muscle values per 100 g fat free solids					
Total H_2O (ml)	337 5	335 3	− 2 2	0 77	—
H_2O_{Cl} (ml)	72 3	60 1	−12 2	1 59	—
(Total H_2O—H_2O_{Cl} (ml)	265 2	275 2	10	1 20	—
Cl (mEq)	8 7	6 8	− 1 9	2 06	—
Na (mEq)	11 4	10 2	− 1 2	1 44	—
Na_x (mEq)	1 1	1 8	0 7	1 81	—
K (mEq)	44 5	43 8	− 0 7	1 05	—
Phosphate (mM)	28 9	28 9	0	—	—
K/P (mEq/mM)	1 54	1 52	− 0 02	—	—

Symbols as in table I

considerably more than the decrease in total water The muscle sodium content decreased slightly Excess sodium increased by 1 6 mEq/100 g FFS The muscle potassium content decreased by 2 8 mEq/100 g FFS or 6 2 per cent of the basal value The potassium/phosphorus ratio was also lowered (— 0 1 mEq K/mM P = 6 6 per cent of the basal value) The muscle potassium decrease cannot be attributed only to loss of extracellular potassium since the total extracellular potassium content in muscle tissue should not amount to more than about 0 3 mEq/100 g FFS

b) The effect of furosemide

The changes in muscle tissue were less pronounced Total water was unchanged before and after the treatment Moderate but not significant decreases were found in chloride content and chloride space Nor were the changes in muscle sodium and potassium content significant and phosphate remained unaltered

TABLE V Combined results in 26 normal subjects Hydrochlorothiazide polythiazide or chlorthalidone during 1 week Difference in water and electrolyte content in muscle tissue before and after drug administration

Per 100 g FFS	Mean difference after—before diuretic	Per cent of basal value	t	Significance
Total H_2O (ml)	−12 1	− 3 5	4 12	***
H_2O_{Cl} (ml)	−21 7	− 29 4	6 95	***
(Total $H_2O-H_2O_{Cl}$) (ml)	+10 5	+ 3 9	2 93	**
Cl^- (mEq)	− 3 13	− 36 1	8 40	***
Na (mEq)	− 1 30	− 18 5	3 03	**
Na_x (mEq)	+ 1 63	+183	7 22	***
K^+ (mEq)	− 2 76	− 6 2	5 73	***
Phosphate (mM)	+ 0 03	0	0 08	—
K/P (mEq/mM)	− 0 10	6 6	6 93	***

Symbols as in table I

TABLE VI Muscle glycogen content in normal man before and after 7 days administration with diuretics Results expressed as g glycogen/100 g wet muscle tissue

Diuretic	n	Mean value		Difference	t	Significance
		Before $\overline{m}$	After $\overline{m}_1$	$\overline{m}_1-\overline{m}$		
Hydrochlorothiazide (100 mg/d)	10	1 30	1 09	−0 21	2 74	*
Hydrochlorothiazide (150 mg/d)	10	1 30	1 29	−0 01	—	—
Polythiazide (4 mg/d)	18	1 34	1 28	−0 06	-	—
Chlorthalidone (200 mg/d)	12	1 27	1 18	−0 09	1 93	—
Furosemide (100 mg/d)	22	1 37	1 19	−0 18	3 38	**

Symbols as in table I

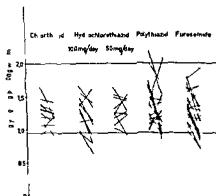


Fig 1 Muscle glycogen determined before and after one week s administration of four different diuretics Normal range of muscle glycogen 0 95 to 2 0 g/100 g wet muscle tissue

4 *Blood sugar and muscle glycogen*

The blood sugar was not significantly changed in any of the series

The results of the analyses of the muscle glycogen levels are demonstrated in fig 1 and table VI The results were irregular with increases as well as decreases of the muscle glycogen content in all the series Values down to 0 90 or less were recorded with all four diuretics, denoting a decrease down to the lower normal limit or below However, the mean decreases were significant only for hydrochlorothiazide 100 mg daily ($p < 0.05$) and furosemide 100 mg daily ($p < 0.01$)

Discussion

A *Electrolyte changes after treatment with hydrochlorothiazide polythiazide, and chlorthalidone (i e diuretics of the thiazide type)*

The effect on body weight indicates that the total body water decreased If total body water is reckoned to represent approximately 60 % of the body weight, the weight loss (about 2 kg) will correspond to 5 4 % of the total body water This exceeds the water loss of 3 5 % which was directly determined in muscle tissue, indicating that a proportionately greater loss of water occurred from tissues other than skeletal muscle

The decrease in the chloride space much exceeded the decrease in total muscle water Assuming that the chloride space is a reliable measure of the extracellular fluid volume, this result would indicate that the muscle cells had gained water as a consequence of the thiazide administration However another explanation could be that a chloride compartment outside the extracellular fluid had been reduced There is evidence that chloride and potassium are distributed according to a Donnan equilibrium, i e the intracellular chloride concentration decreases along with decrease in the concentration of extracellular potassium and vice versa However, the influence of the extracellular potassium decrease on the intracellular chloride concentration should be negligible from a quantitative point of view in the present experiments On the other hand it cannot be ruled out that other chloride compartments e g in the connective tissue accompanying the muscle fibers could be changed

The present investigation demonstrates that diuretics of the thiazide type (hydrochlorothiazide polythiazide and chlorthalidon) cause a decrease of the muscle potassium content of about 6 % in normals In a subject with a total

body potassium of about 3,000 mEq a loss of this magnitude would, if affecting the whole potassium store uniformly, correspond to a potassium loss of about 180 mEq This tallies with the results obtained in balance studies by Sandoe and Olesen (23) Our results with regard to potassium also verify those of Gifford et al (8), Hollander et al (10) and Kaul et al (15), but are not in conformity with those of Talso and Carballo (31) In our study the drugs were administered in larger doses than in most other investigations and the observation period was shorter It is possible that treatment for a longer time may involve a secondary adaptation to diuretics (8) either by increased potassium intake or by reduced excretion of potassium

Rooth and Furst (22) expressed the opinion that the hypokalemia could be attributed to the extracellular metabolic alkalosis, which was measured by them as base excess It is nevertheless, evident from our study, and from that of Sandoe et al (23) that the alkalosis is partly compensated through respiration changes, with the result that the pH is but slightly changed Earlier studies have established that the extracellular hydrogen ion concentration, and not the bicarbonate concentration is the factor influencing the distribution of potassium between the plasma and the cells (24 27)

The increase of excess sodium in muscle tissue indicates that an uptake of sodium takes place in the cells If so, sodium is apparently leaving the extracellular fluid in thiazide treatment not only by urinary excretion but also by redistribution within the body It may seem paradoxical that a strong natriuretic agent should increase, instead of reduce, the intracellular sodium content However, it has been demonstrated by means of isotope dilution that normal subjects, on a sodium deficient diet, lose more sodium from the extracellular fluid than can be attributed to the urinary excretion, which was considered to indicate sodium uptake in the cells (6)

It has previously been established in potassium deficiency that the potassium lost from the cells is replaced by as a rule 2 sodium ions and 1 hydrogen ion per 3 potassium ions (5) With depletion of sodium concomitant with depletion of potassium in rats, also a cellular uptake of sodium could be discerned though small in comparison with the loss of potassium (11)

In the present study, hydrochlorothiazide, polythiazide, and chlorthalidone caused a decrease in the muscle potassium content (—2 8 mEq/100 g FFS) exceeding the increase of sodium (+1 6 mEq/100 g FFS) (table IV) This may indicate an increased intracellular hydrogen ion content, i e the extracellular alkalosis induced by thiazide diuretics may be generated by influx of hydrogen ions into the cells Additional evidence for this is the fact that the alkalosis with thiazide treatment cannot be explained by an increased urinary hydrogen ion excretion (20)

A fall was recorded in the plasma phosphate concentration in all three materials, being significant after treatment with hydrochlorothiazide and polythiazide However, no fall was noted

in the muscle phosphate content, which might indicate that the cells are not subjected to a corresponding loss of phosphate It has earlier been observed in clinical material that no correlation exists between the extracellular phosphate concentration and the muscle phosphate content (1)

B *Comparison between thiazides (and chlorthalidone) and furosemide*

The effect of furosemide on the acid base equilibrium plasma potassium, and plasma chloride was similar to that of the thiazide diuretics The decrease of potassium and chloride concentrations was, however, less pronounced The effect on body weight also resembled that of the thiazides and the rise in plasma protein was even greater, indicating a considerable decrease in total body water as well as plasma volume In other words a strong diuretic effect had been obtained well comparable to that of the thiazide drugs In contrast to the thiazides the effect on the potassium content of muscle tissue was small and insignificant

It has been proved earlier that thiazide diuretics as well as furosemide cause a compensatory rise in the secretion and excertion of aldosterone in normals (14 17 19) As thiazides and furosemide individually give rise to changes that are largely comparable with regard to total body water (body weight) and plasma volume (plasma protein concentration) also the increased aldosterone secretion should be comparable during treatment with both types of drugs The fact that furosemide, in contrast to the thiazides, did not produce a significant loss of potassium repudiates the supposition that aldosterone is the determinant of the extent of potassium loss after diuretics in normals A direct difference in the kaliuretic effect between furosemide on the one hand and thiazides on the other hand, independent of aldosterone, would be a more plausible explanation

C *Muscle glycogen changes*

An investigation of the effect of diuretics on muscle glycogen was motivated by the well known fact that certain diuretics of the thiazide type are diabetogenic (9) Effects of this kind have been reported after treatment with hydrochlorothiazide (33) chlorthalidone (7), and polythiazide (18), though some investigators contend that they are less conspicuous with the last mentioned drug (29) We have earlier demonstrated that patients with juvenile diabetes are subjected to considerable decreases in muscle glycogen (4) Tabachnich et al (30) found a lowered glycogen content in the liver of mice given diazoxide a thiazide without any saluretic effect

With all the diuretics individual subjects showed a decrease in muscle glycogen content to values below the normal range but only in two series (hydrochlorothiazide 100 mg daily and furosemide) was the mean decrease significant Recent studies at our laboratory on normal subjects have disclosed considerable variations in the muscle glycogen content depending on diet and muscle activity Thus variations in these two factors may conceivably have contribut

ed to the inconsistency of the results Still it is possible that the glycogen decrease in some subjects may be due to a direct action on carbohydrate metabolism by the drug administered Individual differences in susceptibility may exist in normal subjects as well as in diabetics

Since the diet and the amount of daily exercise were not standardized, no definite conclusion can be drawn from our results concerning the diabetogenic action of the different diuretics

Summary

Hydrochlorothiazide, polythiazide, chlorthalidone, and furosemide were given to normal subjects daily during one week

Body weight, plasma protein, plasma electrolytes, and blood pH were determined before and immediately after the drug administration period Needle biopsies from *m quadriceps femoris* were performed on the same occasions The muscle tissue was analysed for water, sodium, potassium, chloride phosphorus and glycogen content, neutron activation analysis being used for the electrolyte determinations

Comparable changes in body weight were recorded after the four different drugs Increases in plasma protein concentration were also obtained, being most pronounced in the furosemide series

In all four series hypokalemia, hypochloremia, and alkalosis were obtained These changes were somewhat less pronounced with furosemide

Significant changes in water and electrolyte contents of muscle tissue were found after administration of hydrochlorothiazide, polythiazide and chlorthalidone A slight fall in extracellular water content and a pronounced fall in chloride content and chloride space were recorded Excess sodium, i e sodium outside the chloride space, was increased The potassium content decreased in relation both to fat free solids and to total phosphorus, which was unchanged

After furosemide administration the water content in muscle tissue was essentially unchanged The chloride content and chloride space decreased but not to a statistically significant extent Nor was sodium, potassium and phosphorus in muscle significantly changed

Muscle glycogen decreases were obtained in the majority of the subjects with all the four diuretics, but the mean decrease was significant only with hydrochlorothiazide (100 mg daily) and furosemide

The results are discussed

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From Medical Department A (Head K Brochner Mortensen M D) University Hospital Copenhagen, Denmark

T-max-glucose in Pseudohypoparathyroidism

By

Bent Halver

In 1942 Albright et al (1) suggested the term pseudohypoparathyroidism for a syndrome with clinical findings as in idiopathic hypoparathyroidism but in which parathyroid extract failed to normalize the abnormal values of serum calcium and phosphate This non-reactivity suggested that the disturbance is caused by an increased renal resistance to a normal or even increased production of endogenous parathyroid hormone Furthermore the syndrome includes developmental abnormalities consisting of a short thick set stature, roundness of face and shortness of fingers Since that time more than 70 cases have been reported A review was published by Mann et al in 1962 (7)

Recently, Halver (6) suggested an influence of the parathyroid hormone on the maximal tubular reabsorption capacity for glucose (TmG) A correlation was found between the parameter TmG/GFR that is TmG related to the glomerular filtration rate, and the state of parathyroid function Increased values of TmG/GFR were found in hyperparathyroidism and low values in hypoparathyroidism The existence of this correlation has been confirmed by subsequent studies (to be published)

The purpose of this report is to present a case of pseudohypoparathyroidism in which the diagnosis was first suspected because a state of parathyroid hyper function was suggested by demonstration of high values for TmG/GFR

Case report

A 45 year-old woman was admitted to the University Hospital Copenhagen in June 1964 (Medical Department A) Three years previously an explorative laparotomy was performed because of loss of weight diarrhea and a barium enema suggestive of a tumor of the rectum However no abnormalities were found during the operation Her symptoms persisted and a few months later a diagnosis of thyrotoxicosis was made One year later a subtotal thyroidectomy was performed leaving parts of both lobes After the thyroidectomy she became euthyroid but she developed a paranoid psychosis The paranoia almost disappeared during

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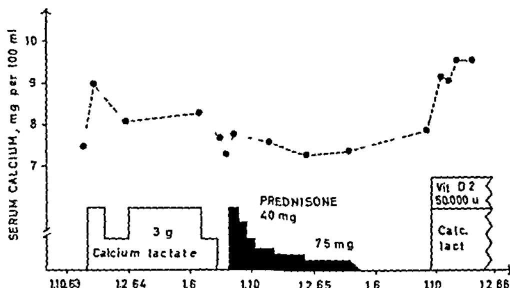


Fig 1 Serum calcium levels in a patient with pseudohypoparathyroidism

treatment with chlorpromazine for 8 weeks Eight months after the thyroidectomy the patient was admitted to the University Hospital with a few months history of slight exophthalmos diplopia and twitches of the facial muscles No determination of the serum calcium level was done before the thyroidectomy In the following months the values ranged from 7 5 to 8 3 mg/100 ml

The patient was a short thick set female with short fingers and a round face Her weight was 66 kg and her height was 157 cm Clinically she was euthyroid The Chvostek and Trousseau signs were negative The examination revealed a paresis of the right superior rectus muscle and bilateral exophtalmus Hertel 24—22) X ray studies of the extremities and of the skull did not show any characteristics of pseudohypoparathyroidism Mentally she was shy and slightly retarded but with no signs of paranoia

ESR 9 mm hour hemoglobin 13 3 g 100 ml serum magnesium 1 5 mEq l serum calcium 7 3 to 7 8 mg 100 ml serum phosphate 3 8 to 4 2 mg/100 ml total protein 6 8/100 ml, albumin 4 65 g 100 ml serum alkaline phosphatase 5 2 King Armstrong units BMR + 10 and − 4 serum protein bound iodine 5 0 μg/100 ml serum lipid phosphate 4 60 mmol/l (normal 2 00—3 45), serum citric acid 193 μmole/l (normal 88—141)

Clinical course

During prednisone therapy (fig 1) the exophtalmos regressed rapidly After 2 months of therapy the Hertel values were 19—17 and 5 months after withdrawal of prednisone the exophtalmos had not relapsed (Hertel 17—16) Following administration of calcium lactate and vitamin D_2 the serum calcium rose to normal values (fig 1)

Clinical investigations

A determination of TmG GFR was performed (the methods have been described previously (6)) and surprisingly high values were found suggesting a state of hyperparathyroidism (table 1) No significant increase in TmG GFR was demonstrable following intramuscular injections of 200 units of parathyroid extract b i d for 4 days Five months later a further increase in TmG GFR was found suggesting a progressive state of hyperparathyroidism The serum calcium level did not rise after ad

TABLE I Determinations of TmG/GFR in a patient with pseudohypoparathyroidism before and after the administration of parathyroid extract and after 5 months without therapy The mean value of TmG/GFR is 1 97±0 09

Duration of period (min)	Diuresis (ml/min)	Glucose in urine (mg/min)	Inulin in urine (mg/100 ml)	Inulin in plasma (mg/100 ml)	Glucose in plasma (mg/100 ml)	GFR (ml min)	TmG (mg/min)	TmG/GFR
9/4 1965								
15	8 4	316	732	48 9	565	126	395	3 13
15	16 4	363	364	46 2	570	129	375	2 91
15	19 8	393	280	45 4	590	122	326	2 67
15	13 0	489	482	44 5	610	141	370	2 62
Mean						130	366	2 83
Following administration of parathyroid extract 200 units b i d for 4 days								
15	10 7	449	660	54 8	656	129	397	3 08
15	18 0	547	444	53 2	663	150	448	2 99
15	20 7	592	353	51 1	653	143	342	2 39
15	15 0	600	472	50 4	678	140	349	2 49
Mean						141	384	2 74
16/9 1965								
15	11 7	197	325	45 5	565	84	275	3 29
15	20 0	314	291	45 8	546	127	379	2 99
15	17 3	310	291	46 1	606	109	351	3 22
15	10 3	273	435	45 5	616	99	334	3 39
Mean						105	335	3 22

ministration of parathyroid extract In comparison four cases of postoperative hypoparathyroidism treated in the same way showed a marked increase of the serum calcium level (Fig 2) An Ellsworth Howard test (4) revealed merely a doubling of the urinary phosphate excretion in spite of a pronounced increase in GFR and hence an increase of the filtered load of phosphate (table II The tubular reabsorption of phosphate (TRP %) was 87 1 87 6 and 89 9 on 3 consecutive days Determinations of TRP during the Ellsworth Howard test gave the same results and these values did not fall noticeably following the administration of parathyroid extract (table II The tubular reabsorption of calcium was 98 9 %

Since glucose interferes with the colorimetric estimation of inulin twelve separate investigations were carried out to determine a conversion factor which was 1 25 % (± 0 16 %) This factor deviates from the one earlier reported (6) The conversion factor previously used resulted in a systematic overestimate of the true values of inulin clearances and TmG With the new factor

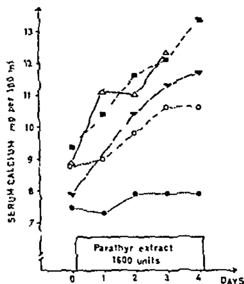


Fig 2 The effect of parathyroid extract on the serum calcium level in four cases of post operative hypoparathyroidism (△ ▼ ■ ○) and in one case of pseudohypoparathyroidism (●)

the normal range of TmG/GFR as measured in 6 normal individuals is 1 97 ± 0 09 (± 2 standard deviation)

All the investigations have been performed with the same batch of parathyroid extract (*Para—thor—mone*[R] 100 U S P *para* thyroid units per ml Eli Lilly & Comp

Discussion

The constant finding of a low serum calcium level and the history of twitching of the facial muscles following neck surgery suggested a diagnosis of post operative hypoparathyroidism However, a state of hyperparathyroidism was strongly supported by demonstrations of high values for TmG/GFR In addition, there was shown to be a lack of response to exogenous parathyroid hormone, as measured by TmG/GFR Hypoparathyroid patients are shown to reveal a significant increase in TmG/GFR following administration of parathyroid extract (6) Only a slight response to parathyroid extract, as measured by TRP, was demonstrated *Among others, Becker et al (2) consider* this to be characteristic of hyperparathyroidism Among 9 hyperparathyroid patients, the greatest decline in TRP after administration of parathyroid extract was 6 8 % Two hypoparathyroid patients, one idiopathic and one post operative, showed declines of 12 0 and 18 6 %

TABLE II Ellsworth Howard test and tubular reabsorption of phosphate (see text)

Time (a m)	Urine phosphate (mg)	Creatinine clearance (ml min)	Serum-phosphate (mg/100 ml)	TRP (%)
8— 9	21	85	4 4	90 6
9—10	25		—	—
10—11	16	—	—	—
11—12	19	75	4 8	91 2
Parathyroid extract 200 units i v at 8 a m				
8— 9	32	115	4 4	89 5
9—10	47		—	—
10—11	42	125	4 0	86 0

The diagnosis of pseudohypoparathyroidism was confirmed by the demonstration of a total resistance to exogenous parathyroid hormone as measured by the serum calcium, and of a relative resistance as measured by the phosphate excretion The clinical appearance of the patient gave a further support to this diagnosis

Usually the serum phosphate concentration is increased in pseudohypoparathyroidism However Sjaastad (9) reported one case with a normal serum phosphate level and quoted 4 cases from the literature with only slight elevations of the serum phosphate levels In the present case the serum phosphate was within normal limits This could not be explained by malabsorption or excessive renal loss of phosphate

The high values of GFR found in the two first TmG determinations are due to the prednisone therapy As shown by Froesch et al (5) prednisone has no influence on the TmG/GFR rate, which is in accordance with the author's experience

A few cases of pseudohypoparathyroidism with decreased glucose tolerance have been described (3 7 8) However, in no case did the patient develop glucosuria following the glucose administration although the plasma glucose levels were well above the normal renal threshold for glucose In the case of Moehg and Gerisch (8) the plasma glucose reached a level of 235 mg per 100 ml but glucosuria did not occur The lack of glucosuria is compatible with the increased reabsorption capacity for glucose in hyperparathyroidism (6)

The finding of a parallel relationship between blood glucose levels and phosphate clearances suggests a competitive inhibition of the tubular reabsorption of glucose and phosphate (references have been cited recently (6)) Based upon this assumption it has been suggested that the influence of parathyroid hormone on the reabsorption capacity for glucose is due to an inhibition of the phosphate reabsorption, which might make a correspondingly larger part of a common tubular transport mechanism available for the reabsorption of glucose (6) However in pseudohypoparathyroidism no inhibitory effect of parathyroid horomone on the phosphate reabsorption exists and still a pronounced effect on the tubular glucose transport is found which is the principal finding in this study This result suggests that the influence of parathyroid hormone on the TmG is not a consequence of an inhibition of the phosphate reabsorption, but more obviously is a direct action of the hormone on the glucose reabsorption mechanism in the tubular cells

Summary

A case of pseudohypoparathyroidism is reported in which the diagnosis was made as a consequence of the finding of an increased tubular reabsorption capacity for gluco e According to recent evidence increased glucose reabsorption is a consequence of parathyroid hyperfunction The mechanism by which the parathyroid hormone may influence the glucose reabsorption is discussed

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√ Book review

Handbok of Physiology Section 2 Circulation Volume III American Physiological Society Editors W F Hamilton and Philip Dow 978 p The Williams & Wilkins Co Baltimore, Maryland 1965

This is the third and final volume in the section on circulation in the American Physiological Society's Handbook of Physiology The volume contains 26 chapters dealing with different aspects of the circulation as an integrated whole, as well as an index for all three volumes As in the first two volumes the authors of the various chapters are among the most prominent in their respective fields and the result is that the accounts are up-to-date and of extreme value It is difficult to pick out a particular chapter, but the introductory chapter by Folkow, Heymans and Neil, which presents an admirable survey entitled *Integrated aspects of cardiovascular regulation*, is of great general interest also Davis' *The physiology of congestive heart failure* Page and McCubbin's *The physiology of arterial hypertension* and Ahlquist's *Effect of the autonomic drugs on the circulatory system*

Developments in the field of the physiology of the circulation have been very rapid in recent years, and new investigations and methods have produced results which have caused us to change our views of fundamental questions Now that the Circulation Section of the Handbook of Physiology has been completed, we have an up to-date, comprehensive (nearly 2 700 pages) work of high class in a clearly arranged presentation dealing with a physiological field of central importance, and with valuable pathophysiological perspectives

By drawing upon a large number of authors — each an expert of the highest class in his own field — and through the fact that the handbook could be brought out as a whole within a relatively short period (1962—65), the editors have been able to produce a very much more up-to-date and competent book than the usual run of text books The work is warmly recommended to physiologists, cardiologists, internists, anaesthesiologists and others

Hans Duner
Stockholm

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From the Central Laboratory (Head P Lous M D) Bispebjerg Hospital and the Medical Department (Heads A Harrestrup Andersen M D and Finn Jensen) Sankt Lukas Stiftelsens Hospital, Copenhagen Denmark

Determination of Peptic Activity and Acid in the Gastric Juice of Patients with Peptic Disease Before and After Administration of Glycopyrrolate

By

Vibeke Bitsch and Marie Kristensen

The role of pepsin and hydrochloric acid in peptic disease is beyond doubt Previously, it was believed that the production of both substances was parallel, but more recent investigations in humans as well as animals, have shown that this is not always so (6 9 12, 14, 16 21) Accordingly there is an increased requirement for determinations of the acid and pepsin concentration in the gastric juice, not least for assessing the effect of various drugs

The object of the present study was to investigate the effect of the anticholinergic drug glycopyrrolate (glycopyrronium bromidum NFN Tarodyl® 1 methyl 3 pyrrolidyl α phenyl cyclopentane glycolate methobromide (H Lundbeck & Co Ltd Copenhagen)) upon pepsin as well as acid secretion in patients with peptic disease

Submitted for publication February 2 1966

Material and methods

The concentration of pepsin and acid was studied in the gastric juice of 10 patients admitted to the Medical Department of the St Lukas Stiftelsens Hospital Copenhagen, for complaints indicating peptic disease These patients ranged in age from 20 to 65 years Only 5 showed fresh ulcer craters In the others any other cause of the dyspepsia than peptic disease was ruled out partly by a thorough history and partly by X ray examination of the biliary tract and colon proctoscopy and tests of pancreatic function Table I lists the exact diagnosis sex weight and age

Radiopgraphic examination of the stomach with contrast medium showed emptying in less than 4 hours in all 10 patients Prior to the examination the patients had fasted for 10 hours and had taken no medicine for 24 hours

Glycopyrrolate

The effect of glycopyrrolate was assessed on the basis of secretion under basal conditions

1—663007 Acta Med Scand Vol 180 4

TABLE IV Pepsin output in mg

Patients	Control period (quarter hour)		Total during 1st hour	After glycopyrrolate (change from control period in % within brackets)			
	2nd	4th		2nd hour	3rd hour	4th hour	5th hour
A B	20 3	11 9	64 4	14 6	14 8	17 6	30 7
K O	20 4	30 6	102 0	28 7	17 2	18 9	23 4
M L	6 8	5 0	23 6	5 5	0 0	0 3	0 4
M D	19 5	22 2	83 4	8 9	0 4	1 0	2 3
C N	25 2	40 0	130 4	33 6	10 5	8 2	1 1
K P	17 4	16 6	68 0	19 4	3 5	12 0	14 3
V E	28 6	26 2	109 6	24 3	8 6	20 0	22 0
B B	7 7	14 5	44 4	12 8	6 2	6 4	6 5
K N	17 8	15 1	65 8	14 7	15 7	8 4	17 3
E T	13 1	14 8	55 8	19 5	9 8	15 5	17 7
Mean	17 7	19 7	74 8	18 2 (÷76%)	8 7 (÷88%)	10 8 (÷85%)	13 6 (÷82%)

The mean pepsin concentration in all 10 patients was 597 mg/l (range 180—1160 mg/l) under basal conditions, and a tendency to increase was not found until 3—4 hours after the administration of glycopyrrolate (table III).

Pepsin output

The pepsin output during the 1st hour averaged 74 8 mg but after administration of glycopyrrolate it fell in all patients. The minimum output was observed during the 2nd hour after administration, averaging 8 7 mg corresponding to a reduction of 88 %. During the 4th hour after administration the pepsin output was still reduced by 82 % (table IV).

Concentration of free acid

Unlike the pepsin concentration the concentration of free acid decreased in all the patients under the influence of glycopyrrolate. The concentration of free acid decreased to 0 in 7 patients, including the two (V. L. and B. B.) who showed an increase in pepsin concentration. The mean concentration of free acid in all 10 patients decreased from 29 2 mEq/l under basal conditions to a minimum of 3 6 mEq/l during the 3rd hour of the test corresponding to a reduction of 88 %. During the last hour of the test the concentration of acid was still reduced by 76 % (table V).

pH in the aspirates

The pH in the gastric juice under basal conditions was less than 1 5 in all the patients but one (M I who while suffering from nausea showed a transient increase. The pH in the fractions lacking free acid — before or after administration of glycopyrrolate is listed in table V. It was only in 12 out of the 40

TABLE V Free acid concentration in mEq/l (if zero pH is given)

Patients	Control period (quarter hour)				After glycopyrrolate (change from control period in % within brackets)			
	1st	2nd	3rd	4th	2nd hour	3rd hour	4th hour	5th hour
A B	38.4	33.6	15.8	14.2	21.0	8.7	16.9	21.7
K O	70.2	46.7	57.6	60.7	44.0	23.6	53.7	35.3
M L	0	0	4.9	3.7	0	0	0	0
	(3.5[1])	(6.2[1])			(4.1[1])	(7.9[1])	(8.4[1])	(8.0[1])
M D	14.2	7.2	16.4	10.0	8.4	0	0	0
						(8.0[1])	(8.1[1])	(7.9[1])
C N	21.0	29.0	23.4	28.6	12.2	1.6	0	0
							(6.8[1])	(7.5[1])
K P	72.1	61.4	54.4	57.8	27.9	2.0	1.0	2.7
V E	42.2	37.1	41.3	44.3	31.8	0	0	0
						(4.8[1])	(4.2[1])	(4.1[1])
B B	33.8	28.3	25.4	43.0	31.0	0	0	0
						(3.7[1])	(4.2[1])	(4.1[1])
K N	30.8	7.7	12.7	11.0	5.4	0	0	8.8
						(3.8[1])	(3.4[1])	
E T	11.7	13.0	23.4	14.0	6.2	0	0	0
						(7.1[1])	(6.4[1])	(4.6[1])
Mean	33.9	26.4	27.5	28.7	18.8	3.6	7.2	6.9
					(−36%)	(−88%)	(−75%)	(−76%)

[1] (pH)

Mean for 1st hour 29.2

TABLE VI Free acid output in mEq

Patients	Control period (quarter hour)				Total during 1st hour	After glycopyrrolate (change from control period in % within brackets)			
	1st	2nd	3rd	4th		2nd hour	3rd hour	4th hour	5th hour
A.B	1.38	1.48	0.66	0.58	4.10	0.55	0.21	0.51	0.89
K.O	1.61	1.54	1.79	2.00	6.94	2.24	1.03	2.79	1.79
M.L.	0	0	0.18	0.10	0.28	0	0	0	0
M.D	0.68	0.36	1.02	0.53	2.59	0.19	0	0	0
C.N	1.39	1.31	1.12	1.23	5.05	0.69	0.03	0	0
K.P	1.30	0.92	0.98	0.92	4.12	0.56	0.01	0.01	0.03
V.E	2.19	1.97	2.27	1.64	8.07	1.43	0	0	0
B.B	1.59	0.81	0.72	1.08	4.20	0.65	0	0	0
K.N	0.75	0.25	0.34	0.26	1.60	0.17	0	0	0.19
E.T	0.22	0.25	0.44	0.20	1.11	0.13	0	0	0
Mean	1.11	0.89	0.95	0.85	3.80	0.66	0.13	0.33	0.29
						(−83%)	(−97%)	(−91%)	(−92%)

fractions aspirated under the influence of glycopyrrolate that the pH exceeded 4 5

Acid output

During the first hour of the test an average of 3 80 mEq free acid was secreted During the 2nd hour glycopyrrolate gave a maximum reduction of acid output, to an average of 0 13 mEq/hour, corresponding to a reduction of 97 % During the 5th hour the acid output was still considerably reduced, i e an average of 0 29 mEq/hour (table VI)

Side effects

Dryness of the oral mucosa was noticed by 9 out of the 10 patients, but only 2 (K O and V E) found it to be very severe and unpleasant

As for other side effects, one patient (M D) experienced oppression accompanied by an increase in the pulse rate

Discussion

While the ability of glycopyrrolate to inhibit the acid secretion in patients with peptic disease is well known (1 2 5, 7 19 22 30 31), its influence upon the secretion of pepsin appears to have been studied by one author only (18) After administration of 4 mg glycopyrrolate by mouth to 5 patients with dyspepsia and hypersecretion, Moeller (18) found a reduction in the pepsin output of only 67 7 % as compared with our 88 % On the other hand the reduction in acid output was 97 % in both investigations The pepsin concentration, which was analysed by the method of West, is not stated

The results of investigating the influence of other anticholinergics upon the pepsin and acid output are meagre and in some cases divergent from ours After administration of probantheline bromide, 50 mg by mouth, Ronsky and Skalá (21) found no change in the volume or secretion of acid, although the output and concentration of pepsin, determined by the method of Placer, was reduced by 40 % and 39 % respectively

A correlation between the output of acid and pepsin (method of Mett) also appears to be entirely lacking under the influence of pentapiperide methyl sulphate (29)

In the present study the findings were dominated by the reduction caused by glycopyrrolate in the volume of gastric juice In addition, the concentration of free acid fell in all patients in 7 even down to zero The average concentration of pepsin remained unchanged, apart from a tendency to an increase during the last hour of the test On the other hand, there was a marked scatter in the alterations which the individual patients showed in pepsin concentration which, unlike the acid concentration, never reached zero A preserved secretion of pepsin, despite the attainment of anacid gastric secretion during treatment with anticholinergic (poldine methyl methosulphate), has previously been reported by Lawrie et al (16) in a patient with Zollinger Ellison s syndrome

Silver et al (26), studying the response of 10 patients to i v injection of methantheline bromide found no scatter in the pepsin concentrations (method of West)

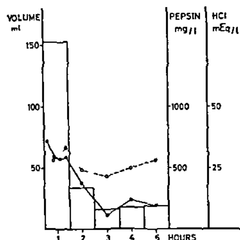


Fig 1 Mean acid concentration (●—●) pepsin concentration (○— —○) and volume (columns) in 6 patients with *peptic disease in the duodenum* First hour under basal conditions the following 4 hours after administration of glycopyrrolate (Tarodyl®) 1 mg i m

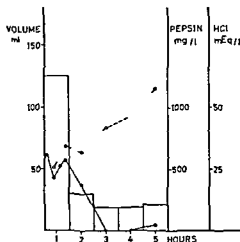


Fig 2 Mean acid concentration (●—●) pepsin concentration (○— —○) and volume (columns) in 4 patients with *peptic disease in the body of the stomach* Fist hour under basal conditions the following 4 hours after administration of glycopyrrolate (Tarodyl®) 1 mg i m

All their patients had duodenal ulcer, while ours had peptic disease either in the body of the stomach or in the duodenum When considering only the results in the 6 patients with duodenal and prepyloric peptic diseases which, from a secretory point of view are identical (17), our results are in keeping with Silver et al s in respect to the *fairly unchanged concentration of pepsin* as well as in other respects (fig 1)

The influence of surgical vagotomy upon the concentration and ecretion of pepsin has been studied in patients with duodenal ulcer (4 8) Borg and Borgstrom (4) found a reduction which was not observed by Gillespie and Bowen (8) if the acid output remained in excess of 10 mEq/30 minutes

The remaining 4 patients who had gastric ulcers (V E B B K N, and F T) showed a divergence in the mean concentration of pepsin and free acid (fig 2)

Despite the small size of our series we believe that our results support the theory that the classification of peptic diseases into types according to whether they affect the body of the stomach or the pylorus duodenum, corresponds to a different variation in the pepsin hydrochloric acid concentration under the influence of various agents

Under basal conditions the concentration of pepsin is the same in normals and in patients with gastric or duodenal ulcer (4, 11, 28)

The mechanism which stimulates the secretion of pepsinogen is far from being elucidated In addition to a continuous basal secretion, the secretion of pepsinogen is initiated by way of the vagus nerve and under the influence of a hormone presumably gastrozymine It

has not been ascertained whether motility of the antrum causes the release of, or merely accompanies, the hormonally conditioned secretion of pepsinogen (10, 23).

In this connection it should be mentioned that glycopyrrolate does not inhibit antral mobility (20) as much as older anticholinergics. This is perhaps the explanation of the difference between the results found by authors who have used older anticholinergics and our own results.

The secreted pepsinogen is converted, under the influence of protons, to pepsin at a pH lower than 5.4. The conversion is rapid and complete at pH 2.

Irreversible denaturation of pepsinogen and pepsin in base takes place at pH 12 and 7 respectively (11, 24). In the present study the pH in the anacid secretions was determined as a maximum of 8.4. Therefore, denaturation of pepsinogen is out of the question. (The greatest peptic activity was measured, e.g., in anacid juices (fig. 2).) The conversion of pepsinogen to pepsin did not take place until during the analysis in the case of these specimens. Therefore, the determined peptic activity represents the output of pepsinogen. On the other hand, it is possible to ascertain the peptic activity in the stomach by comparing the pH and pepsin concentration.

The peptic activity determined at pH 1.9 is a rough measure of the pepsin concentrations, as recent chromatographic investigations have shown that in actual fact there are 4 different pepsinogens and pepsins, having different pH optima (25).

Variations in the concentration of pepsin under anticholinergic influence cannot be explained by simultaneous alterations in the volume of secretion neither in the individual patients nor in each of the two groups. After administration of atropine to rats with ligatures around the pylorus, Hirschowitz (12) — unlike Singh et al. (27) who used oxiphenonium bromide — found the concentration of pepsin to increase owing to a reduction in the volume of gastric juice. Atropine reduced the synthesis of pepsinogen and even more its output. It was not until 2—5 hours after the administration that the overfilling of the chief cells with pepsinogen granules resulted in a high concentration of pepsinogen in the gastric juice. The doubtful increase that we found in the mean pepsin concentration in all the patients may be due to similar phenomena.

There was no correlation between the alteration in the concentration of pepsin on the one hand and the patient's weight, degree of side effects, or attainment of anacid gastric juice on the other. All 10 patients received, regardless of body weight, 1 ml (1 mg/ml) glycopyrrolate intramuscularly.

Summary and conclusion

The relationship between the concentration and secretion of pepsin and hydrochloric acid in the gastric juice was investigated.

In 10 patients with peptic disease the gastric juice was aspirated continuously over a period of 5 hours – the last 4 hours after administration of glycopyrrolate (glycopyrroni bromidum NFN, Tarodyl®), 1 mg intramuscularly

Glycopyrrolate reduced the volume by 89 %, the acid output by 97 % and the pepsin output by 88 % The acid concentration fell in all 10 patients An anacid gastric juice was attained in 7 patients The average concentration of pepsin was unchanged, but with a tendency to increase during the last hour of the test As far as the individual patients are concerned there was a marked scatter in the alteration in the concentration of pepsin unlike that of acid

A possible explanation is a different localization of the peptic disease an increasing concentration of pepsin when it is localized to the body of the stomach (4 patients) and unchanged pepsin concentration when it is localized to the duodenum (6 patients)

It is for continued studies to decide whether this assumption is correct

Acknowledgement

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From the Medical Department B and Cardiovascular Laboratory (Head A Tybjærg Hansen), Rigshospitalet University Hospital Copenhagen Denmark

Complications in Transseptal Left Heart Catheterization

By

O Lindeneg and A Tybjærg Hansen

Transseptal left heart catheterization has within a few years proved valuable as a routine diagnostic method (11) The interatrial septum is punctured with a needle, gauge 17—20 (2, 7, 15) and a radiopaque catheter no 9—7 passed over the needle from the right atrium to the left atrium

The most common complication has been puncture of the atrial wall to the pericardial cavity and to the aortic root (5, 10) Since this may lead to hemopericardium and cardiac tamponade, it is important that such unintended punctures are recognized

Furthermore the needle may cause lacerations and perforation of the catheter even when a leading stylus is used (12 19) and emboli with fragments of the catheter have been reported

During intracardiac puncture the needle may slide off and cause lacerations with disturbances of conduction, perforation of the wall and persistent interatrial septal defect (10, 16 17) The risk of thrombus formation in the heart (14) and embolus later on is thus augmented

The big needle and catheter may dislodge a preformed mural thrombus in the left atrium (10), seen especially in patients with mitral disease and auricular fibrillation

The greater part of these major complications is in some way related to the use of a big needle We therefore have modified our technique as described below in order to reduce the risks mentioned

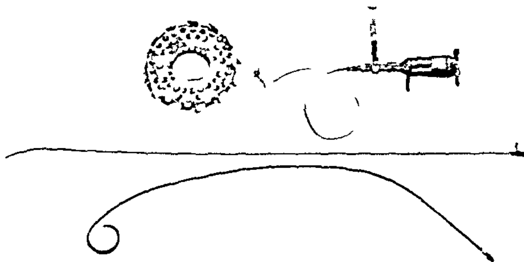
Methods

The technique used hitherto in this laboratory (11) is very similar to the technique originally described by Ross (15) the interatrial septum being punctured with a gauge 17—18 needle introduced with a blunt steel stylet

We have changed to a thin needle 0 75 mm in outer diameter and 0 40 mm in inner diameter The needle is 82 cm long and is connected to a capacitance manometer (8) by means of a 100 cm long nylon tubing with an inner diameter of 0 8 mm To ensure an air free pressure transmitting system all parts are boiled separately and connected without being taken out of the water (9) (fig 1)

The system has been tested for damping and frequency response by a square-wave

Submitted for publication February 23 1966



F g 1 Teflon ca heter s eel gu de and dev ce for pressure measurement The t n needle an l the nylon ca heter are bo led n the conta ner above



F g 2 Tracing of the response to a square wa e pressure variat on

variat on of pressure f g 2 and accord ng to the response is class f ed as a nearly over-damped system 8 the degree of damping being 0 55 0 60 with a natural fre quency of 37 c p s securing an und s orted pressure wave measurement f g 3

The needle is introduced in a steel gu de tub ng wh ch is 81 cm long and has an inner d ameter of 0 8 mm The gu de has a blunt t p and its d stal part is curved in the ind vidual case after the s ze of the r ght atr um

With the needle retracted the gu de is inserted in a no 8 rad opaque teflon catheter wh ch is 80 cm long When the g de has been ad anced almost to the t p of the catheter in the right atr um the gu de and catheter are rotated counter clockw se unt l the t p faces the interatr al sep um n the reg on of the fossa oval s

The steel gu de is next f xed gen ly aga n t the septum and the atr um s punctured w th the needle dur ng pressure record g f g 3) When the pos on in the left a rium is ascerta ned the gu de is ad anced t the t p of the needle and after th s the teflon catheter is pushed forward After removal of the gu de and the needl the ca he r is emptied to prevent embol l an l l f heart catheterizat on is perform ed Should per card al puncture occur f 4 as recogn zed by a drop in pressure and nonal e pressure waves the needle and then the gu de and the catheter are w h awn a d nteratrial septal puncture is a temp ed n ano her pos t on If the need e pene ra es n sol d t ssue in its f ee len h of 10 mm

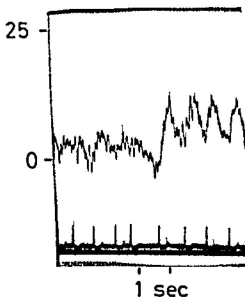


Fig 3 Pressure readings during interatrial septal puncture Case 7031 The rise in pressure and the shape of the pressure waves indicate left atrial entry

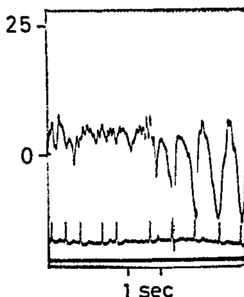


Fig 4 Pressure readings during pericardial puncture Case 7031 The drop in pressure and the negative pressure waves indicate pericardial entry

without entering the left atrium it is suspected that preformed thrombus exists at the site of puncture and transseptal heart catheterization is given up

Material

Interatrial septal puncture was attempted with this technique in 40 cases In 38 cases the left atrium was entered while this failed in 2 cases

In one case with mitral disease and auricular fibrillation the needle penetrated into solid tissue without entering the left atrium during puncture in 2 different positions The other patient had primary pulmonary hypertension and the needle could not be forced through the septum in 2 positions A third attempt was done in a lower position and the pericardium was then punctured

In 38 cases the left atrium was punctured but in 2 cases the catheter could not be advanced because of a tough interatrial septum

In one case of mitral disease the left atrium was punctured in 2 positions but it was not possible to push forwards the guide and investigation was given up In the other case of aortic stenosis and coarctation of the aorta the pericardium was punctured in a third attempt

Transseptal heart catheterization was performed in 36 cases Thirty two patients had valvular heart disease 2 had innocent murmurs one had hypertrophic myocardiopathy and one had coronary arteriolumunal aneurysm Pericardial entry occurred in 3 of these patients with mitral disease

In one case the right atrium was enlarged and in the two others the heart was displaced to the right The pericardium was punctured three times in one case and twice in two cases before the left atrium was entered

In no case of pericardial puncture were there signs of pericardial hemorrhage by stethoscopy ECG or X ray and in one case there was no blood in the pericardial cavity during valvulotomy one week later

Besides pericardial puncture one other complication occurred

A 56-year-old woman with a history of fainting and unexplained hypotensive periods developed hypotension during an otherwise uncomplicated left heart catheterization The condition did not become stable till 16 hours later following treatment with Aramine®

Discussion

Unintended puncture of the atrial wall complicating interatrial septal puncture is mainly due to enlarged right atrium, abnormal position and rotation of the heart (11) or to cranial displacement of the left atrium in severe ventricular hypertrophy (5)

In a large series (1, 5, 6, 10, 13, 18) puncture of the atrial wall to the pericardium or the aorta was observed in 32 of 949 cases and in some materials (1, 18) blood was unexpectedly found in the pericardium later during surgery In these 32 cases major bleeding courrred in 9 causing cardiac tamponade in 6 cases (19 %) of which 3 (9 %) were fatal With such high frequency of cardiac tamponade requiring immediate drainage of the pericardial cavity it is equally important that untoward puncture of the atrial wall is recognized and that measures are taken to lower the risk of major bleeding

It is our experience that unintended puncture of the pericardium and the aortic root can always be recognized when puncture is done during pressure recording With the technique here described continuous pressure tracings are easily obtained and pressure control during puncture has proved to be reliable

As to the high risk of bleeding when the pericardium or the aorta is entered, there is but little doubt that this is due to the big needles and catheters which are pushed through the wall

In more than 800 suprasternal punctures of the aorta and the left atrium with a 0 7 mm needle, we have seen no major bleeding and minor bleeding in only 20 cases in this laboratory (5) Brockenbrough et al (3) performed the interatrial septal puncture with a needle, gauge 18 with a 1 5 cm long tip gauge 21, and no bleeding occurred in 156 punctures

In this material pericardial entry occurred 9 times in 5 cases and no signs of bleeding appeared The steel guide fixes the direction and the position of the needle and as in suprasternal puncture it is unlikely that lacerations can occur

The steel guide never penetrates the wall of the catheter even when forced through the curved catheter at high speed, and the needle remains retracted in the guide until this has passed the tip of the catheter Perforation of the catheter wall (12) or embolism with fragments of the catheter (4, 10) is thus prevented

In one case of mitral insufficiency the needle penetrated into solid tissue without entering the left atrium Mural thrombus was suspected and further transseptal investigation was given up No embolic complications occurred

Summary

Transseptal left heart catheterization carries a certain risk which appears to be mainly due to the big needles which

are often used A technique for interatrial septal puncture with a thin needle is described, and measures to recognize and to prevent the major complications are discussed In a series of 40 patients where this procedure has been used no major complications were seen

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From the Medical Clinic (Head E Ask Upmark M D) University Hospital Uppsala Sweden

The Treatment of Restless Legs

By

ANDERS PARROW and IVAR WERNER

By restless legs (R L) we mean a syndrome of ill defined discomfort in the legs The patients usually complain of a peculiar creeping or crawling sensation rarely painful or described as pure pain, most frequently localized in the lower legs but sometimes in the arms The symptoms always appear at rest and are relieved by movement The patient feels an irresistible need to move his legs, and as the discomfort often starts a short time after the patient has gone to bed, this will prevent the patient from falling asleep The syndrome was described early but did not attract much attention until the last decades after the works by Ekbom in 1945 (6)

The etiology of the syndrome is still unknown (10) A correlation between R L and sideropenia has been shown (1 7 13), but sideropenia with or without anemia occurs only in a minority of the patients just as only a small number of patients with sideropenia complain of R L In spite of the fact that the distress is of completely benign character it often causes the patients serious trouble especially by impeding sleep

Many forms of treatment have been tried Ekbom (6) introduced therapy with vasodilators The discovery of the relationship between R L and sideropenia led to the introduction of iron, which when given intravenously often has a very good effect Nordlander (13) found intravenous iron to be effective also in patients without sideropenia He was also able to show the good effect of other high molecular substances, such as high molecular dextran During the last 10 years we have used high molecular dextran as standard treatment Because of the discussion in the literature we have for the last 5 years systematically followed our patients with R L in order to evaluate different forms of treatment and especially the effect of dextran

Material

Eighty nine patients 25 men and 64 women have been treated They are all the patients treated for R L by us during the years 1960 to 1964 The age distribution is shown in

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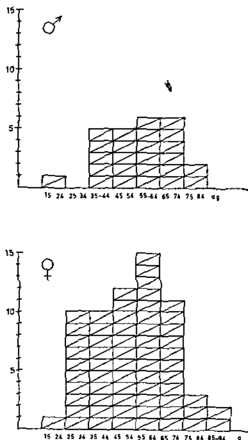


Fig 1 Age distribution

fig 1 The majority of the patients did not suffer from any other detectable disease but sought medical advice for their R L Among the others there was a variety of diagnoses without any apparent overrepresentation of any special disease In 8 patients the hemoglobulin value or hematocrit or serum iron value are missing Of the remaining 81 patients 9 had sideropenic anemia (Hb less than 11 5 g % Hct less than 37 %) and 12 sideropenia without anemia (serum iron less than 60 μ %) Only 2 patients had asymmetrical R L 2 had prevailingly painful sensations 3 patients had their *sensations in the arms as well as in the legs* and one patient complained of sensations only from his arms In most cases the ailment has been intensive and longstanding — for several years — but as a rule we have no exact information about the age of onset *The majority* of patients had been treated with various drugs before seen by us and were actually in most cases referred because of resistance to therapy

Therapy

Most patients who had earlier undergone treatment had received vasodilator drugs or sedatives such as barbiturates meprobamate and klopoxide (Librium®) Untreated patients were usually given vasodilator drugs for instance inositolnicotinate (Hexanicit®) tolazolin (Vasodil®, Priscol®) Ferrigen (Astra) an iron carbohydrate complex in 2 % solution was used for iron therapy the dose given being 5 ml For dextran treatment a dextran fraction with $\bar{M}_w$ 153 000 (Pharmacia) in 10 % solution was used usually 20 ml intravenously A number of patients were also given Ph 1000 (a mixture of equal amounts of the sulphonates of enantaldehyde and furalhyde) (Pharmacia) in tablets of 100 mg 3 times daily

Results

The results are shown in table I Fifty five out of 64 patients (86 %) became completely symptom free after treatment with intravenous dextran Some patients recovered completely already after 1 injection of 20 ml, some patients not before 3—4 injections were given The duration of the remission varied between some weeks and more than two years Four patients improved but were not completely free from symptoms, and five patients did not improve at all, in spite of repeated injections (maximum 4) Among these patients there was a man with discomfort solely in the arms (he was later completely cured by librium and valium)

TABLE I Results of treatment

Therapy	No of pat.		Effect of therapy		
			+	(+)	—
Dextran	14	♂	12	1	1
	50	♀	43	3	4
Intravenous iron	9	♂	7	1	1
	21	♀	13	2	6
Vasodilator drugs	17		0	3	14
Ph 1 000	19		1	10	8

+ = symptom free (+) = improved but not symptom free — = no effect

Twenty out of 30 patients treated with intravenous iron were completely free from symptoms (67 %) The ordinary dose has been 5 ml intravenously after an initial test dose of 2 ml The effect was usually encountered after 2—3 injections (the test dose included) As a rule the sideropenic patients, when symptom free by intravenous iron injections continued with peroral iron preparations As for the dextran treated patients, the duration of remission varied between some weeks and more than 2 years Three patients improved but were not completely symptom free and in 7 patients there was no effect at all All of these last 10 patients were non sideropenic All the sideropenic patients treated with iron intravenously became completely symptom free, but only one half of the non sideropenic patients 11/23 became *symptom free* on intravenous iron injections

All the sideropenic patients treated with dextran (11 patients) became completely free from symptoms

The treatment with vasodilating drugs was much less effective None of the patients became completely symptom free, three improved, and in 14 there was no demonstrable effect

Ph 1,000 had also only a moderate effect *One patient became free from* symptoms 10 improved, and in 8 patients there was no effect at all

Complications

During all the time that we have used high molecular dextran (about 10 years with over 600 injections in 250 patients) the following complications have occurred

1 A 51 year-old man suffering from bronchial asthma developed urticaria the day after the second injection However, some days before he had also started sulphonamide therapy

2 A *50 year-old man* experienced a short lasting nausea when about 14 ml of dextran had been injected

3 An 85 year-old woman who experienced nausea during the injection

4 A 62 year old woman, who after the second injection of dextran experienced a sensation of weakness lasting about 15 minuts, after which she completely recovered After the third injection a few days later she experienced the same sensations but this time more accentuated She was dizzy and had difficulty in standing After one hour's rest she recovered completely

5 A 39 year-old woman, who 6 months earlier had been given dextran intravenously with excellent effect During injection on account of a relapse, she showed blood pressure fall and circulatory arrest She recovered spontaneously after about 30 seconds Four days later she was in perfect health

Discussion

The etiology of R L has been very much discussed during the last years but must still be considered unknown (2, 3, 4, 5 7, 8, 10, 14) It is not our intention to give a complete review of the problem in this article However considering our own experience, we want to stress some points

Sideropenia is common among patients suffering from R L Among our 81 patients there was evidence of sideropenia in 21 cases, which is in good agreement with the frequency given by Ekbom 13/34 (7), 16/48 (8), 19/77 (9) It also corresponds well with the frequency of sideropenia in comparable patient materials It might possibly be presumed that the real percentage of sideropenia is still higher, as we nowadays know that neither Hb nor Hct nor serum iron values give a reliable interpretation of the occurrence of sideropenia (18)

The striking effect of iron intravenously does not prove sideropenia to be the etiology of R L, because relief is often seen on such small doses of iron that a fully developed sideropenia hardly can be considered improved to any noteworthy degree Further, in our material, there are patients who in all probability were not sideropenic but nevertheless reacted immediately to iron therapy as well as certain sideropenic cases, who were quite symptom free after dextran treatment

In the earlier literature (cited by Ekbom (9)) R L was usually considered to be a hysterical manifestation Gorman et al (11) studied 27 patients with R L syndrome and found that the symptoms of R L were most commonly associated with anxiety or depression In spite of the fact that we have not given our patients a thorough psychiatric examination, we, like Ekbom, do not feel that R L is the result of an underlying psychic disorder

Brenning (3, 4, 5) uses the term *molimina crurum nocturna*, with which he denotes R L, nightly cramps in the legs burning feet and other sensations He considers them all different manifestations of a common underlying disturbance However, as for R L and night cramps it is to be noted that therapy with dextran or iron does not influence the leg cramps, while quinine or chloroquine preparations, which are very effective against cramps in the legs, have no effect on R L (17) For this

and other reasons we do not consider these two disturbances to be of identical origin

The effect of high molecular dextran is strikingly good in our patients The mode of action is unknown One possibility is that there is a direct effect of the dextran molecules on the distribution of red corpuscles and plasma in the microcirculation Another possibility is that the effect is due to the histamine liberating properties of the dextran fraction The same mechanism may also be responsible for the effect of intravenously injected iron and 48/80 (cf 15)

The effect of vasodilator drugs was only moderate Out of 17 patients none became symptom free and only 3 improved This percentage of failure may be too high as probably many cases with moderate symptoms have tried this therapy with good results and were thus never referred to us Anyhow our results with vasodilator drugs do not agree with those obtained by Ekbom and by Lindqvist (12)

Our experience with Ph 1 000, introduced by Brenning (5) was rather limited The results do not seem very favourable, as only about 50 % were improved and only one out of 19 completely cured

Ekbom (10) reported very good results on R L with diazepan (Valium®) and klopoxid (Librium®) Our experience with these drugs are limited but the results are favourable In some patients however side effects, mostly drowsiness forced the patients to discontinue the treatment and in some others there was no effect at all

As for complications with dextran therapy, it must be considered that they were encountered in a much larger material than that presented here The only complications of significance were Nos 4 and 5 During the last four years we have encountered no complications at all which probably is due to the fact that during this time we used a slower rate of injection If the injection of high molecular dextran is made slowly and under careful observation of the patient we consider the risk of complication very small

Summary

Eighty nine patients have been treated for restless legs with intravenous injections of high molecular dextran in 10 % solution, intravenous injections of iron vasodilator drugs and a mixture of aldehyde sulphonates

Twenty one patients had sideropenia (anemia and/or low serum iron value) Fifty five out of 64 patients (86 %) were symptom free after 1—3 injections of 20 ml dextran The effect persisted for some weeks up to more than two years

Sideropenic patients were improved in the same percentage as nonsideropenic patients

Twenty out of 30 patients were completely free from symptoms after intravenous injections of iron All the sideropenic patients were symptom free, but only one half of the non sideropenic patients were symptom free on iron treatment

None of 17 patients were symptom free on vasodilating drugs, but 3 had improved

One out of 19 patients treated with Ph 1,000 was symptom free, 10 had improved

Complications of dextran therapy are rare, when appropriate technique of injection is used

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From the Department of Medicine (Head Jan Waldenstrom, MD) and the Department of Bacteriology (Head Sten Winblad MD) University of Lund Malmo General Hospital Malmo Sweden

Clinical Significance of Chronic Biologic False Positive Wassermann Reaction and "Antinuclear Factors"

By

STIG BERGLUND and MATS CARLSSON

Wassermann s reaction was first described at the beginning of the century It is a complement fixation reaction in which cardiolipin extracted from normal mammalian tissues usually beef heart, is used as an antigen It is known that Wassermann s reaction may be positive even in cases where there is no reason to suspect syphilitic infection The TPI test introduced in 1949 by Nelson and Mayer provided a possibility of distinguishing between false positive and true positive Wassermann reactions

Two types of false positive reactions were discerned an acute one and a chronic one The former type which lasts only for some months occurs in a variety of infections or conditions with tissue destruction e g measles primary atypical pneumonia mononucleosis and myocardial infarction The latter type of reaction may last for years Subjects with this chronic type of reaction are known in American literature as chronic biologic false positive reactors (BFP) They are often apparently healthy inquiry into their personal history reveals no known precipitating factors and their positive Wassermann reaction persists for months and years Interest in this group increased after it had become known that the frequency of chronic BFP reactors is higher among patients with SLE than in the population in general

In recent years chronic BFP reactors have received much space in the literature (3, 6 7 8 14 15 16 17 18) and a positive correlation has been suspected between the chronic BFP-reaction and autoimmune conditions such as SLE Hashimoto s thyroiditis and Sjogren s syndrome Harvey (7) for example reported a study of 192 chronic BFP reactors discovered mainly at examination for a health certificate at prenatal clinics and at medical examination for military service In 2 of these 192 subjects the primary examination also revealed SLE During the observation period ranging from 2 to 25 years

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(average 12 years) Hashimoto s thy roid tis developed in 3 and SLE in a further 12 It was also noteworthy that all the pat ents with SLE or Hash moto s disease were females

Material and methods

The term false pos t ve Wasserma n reac tion denotes a pos t e complement f xat on react on w th card ol p n (Wassermann reac t on) w thout a s multaneous ant comple mentary serum t ter w th or without pos t ve flocculat on tests (Me n cke Kl ne Kahn) n assoc ation w th a negat ve TPI test (Nelson test)

The cl n cal mater al cons sted of 87 pat ents (59 women and 28 men) sat sfy ng the abo e cr ter a The cases were collected from the 1953—63 files of the Department of Bacteriology Malmo general hosp tal Most of the pat ents had been cared for at the hosp tal where rout ne exam nat on on adm ss on ncludes the Wassermann reac t on Therefore the preponderance of females cannot be ascr bed to any par cular select on of the mater al The ser es d d not include a few pat ents w th a pos ti e Wassermann react on negat ve TPI test strongly suspected syph l s n the r h s ory and treated in an early stage w th pen c ll n

Of these 87 pat ents 28 15 men and 13 women had been exam ned only once v th Wa sermann s reaction In 35 (7 men and 28 women the react on at the last follo v up had st ll been pos t ve We dec ded o exam ne

TABLE I Dura on of false pos e Was ermann eact on n all 87 cases (acu e and chron c)

	♂	♀
Durat on unknown	12	10
Du a on < 6 mon hs	9	17
Dura on > 6 mon hs	7	32
Total	28	59

these 28 and 35 pat ents with seri m prote n electrophoresis coagulat on stud es and sero-log cal tests nclud ng Wassermann s reac t on n the hope that t vould allow est ma t on of the pat ents state of heal h as ell as reveal any further serum abnormal t es The rema n ng 24 with a negative react on at the latest control exam nat on ere not exam ned further but judged retrospect ely from their prev ous records

The material was d ded nto 3 groups (table I) *The fir t group* cons sted of 22 pa t ents w th a false pos t ve Wassermann reac t on of unknown durat on *the se ond g oup* of 26 w th temporary false pos t e react on i e a durat on of less than 6 mon hs *the th d g oup* of 39 w th a false pos t e react on for more than 6 months e correspond ng to Amer can ser es of chronic BFP reac ors

The first group was made up of 12 men and 10 women F ve of the men and 2 of the women had d ed before the beg nn ng of the present follo up exam nat on Se en men and 4 women could not be traced Four women refused to cooperate None of the 7 pat ents who had d ed had had any collagen d sease It s l kelv that none of the other pat ents had any collagen d sease e her but the nformat on ava lable about most of them was ncomplete

The second group cons sted of 9 men and 17 women One of the men had heuma o d arthrit s and one woman had l ve c rrhos s F e pat ents were exam ned v h extens ve serology by us the find ngs were no mal except for an ncreased thyro d an body t ter n one man and one woman The diagnoses n th s group ar ed w d ly mostly acute infect ons and were unrelated to any known collagen d sease The g oup was too small to allow any conclus ons abou the sex d st but on

Results

Chron c b olog c false pos ti e react ons

This group is of interest ma nly be cause chron c BFP reactions are common

TABLE II Material of 39 patients with chronic BFP reaction

	♂	♀
Dead	3	3
Followed up by us	3	19
Not followed up by us	1	10
Total	7	32

in SLE, in which frequencies of 10 % to 30 % have been reported (1, 4, 5, 10 11, 13) We therefore expected to find some cases of clear cut or suspected collagen disease in our BFP series Another reason why this group was of interest was that it provided an opportunity to study whether a chronic BFP reaction in an apparently healthy person is a precursor of collagenosis If it is, it would be of clinical interest

At the beginning of the observation period one of the 39 BFP patients, a woman had clinically certain SLE another had myxedema and a third had rheumatoid arthritis During the observation period which was on the average 5 years (range 1—14 years) clinically certain SLE was diagnosed in one woman and was suspected in another

The distribution of the 39 BFP patients is given in table II Of these patients 6 (3 men and 3 women) had died before the beginning of our follow up examination Of the remaining 33 patients, 28 (4 men and 24 women) were invited to take part in the follow up examination 22 (3 men and 19 women) accepted and 6 declined Two women could not be traced The remaining 3 patients, all women were not invited to take part in the examination because the Wassermann reaction had been negative at the last examination They were nevertheless regarded as chronic BFP patients because they had for several years had a false positive Wassermann reaction One of these 3 women at the beginning of the observation period had already had SLE with certainty and Leonhardt found that false positive syphilis reactions of chronic type in SLE sometimes disappear temporarily, possibly because of a decrease in the activity of the disease

The BFP group showed a striking predominance of women 32 of the 39 patients were females Of the 6 patients who had died, one was a woman with malabsorption and enlargement of the liver and spleen, but without clear cut signs of collagen disease In the remaining 5 who died the following diagnoses had been made lymphatic leukemia, encephalomalacia and coronary sclerosis, cardiac decompensation and recurrent thrombosis bilateral ovarian cancer and cancer of the body of the uterus myocardial infarction and stenosis of the aortic valve with endocarditis and bronchopneumonia Of the remaining 11 patients who were not re examined by us one was a woman with a firm diagnosis of SLE *with LE cells and one was a woman* with myxedema which had not been thoroughly investigated

The other 9 patients had varying diseases unrelated to collagenosis The diagnoses of these 9 patients were myasthenia gravis slight intermittent joint pains Buerger s disease benign essential hypertension with bronchial

asthma and mental deficiency, pregnancy and (later) uterine bleeding, pregnancy, diabetes mellitus, BFP reaction in an obviously healthy woman and finally, pulmonary tuberculosis with bronchial asthma, bronchitis and emphysema

Twenty two BFP patients were examined by us regarding their serologic reactions, serum protein pattern and coagulation status (coagulation studies performed at the Coagulation Laboratory, Malmo General Hospital, Malmo (Head I M Nilsson, M D))

The serological studies included tests for syphilis, Bunnell's reaction, tests for demonstrating cold agglutinins, anti streptolysin titer (AST), tests for RA (including sensitized sheep blood cells, tannin treated sheep blood cells coated with human gamma globulin that had been heated to 63° C, and acrylic plast), antinuclear factors (ANF), anti staphylolysin titer (ASTA) and tests for demonstrating thyroid antibodies Investigation of the patient's coagulation status included determination of the clotting time in glass tubes and in plastic tubes Duke bleeding time, platelets, thromboplastin one step, recalcification time, P & P, factor V, anticoagulants, antithromboplastin and antithrombin In 9 (1 man and 8 women) of these BFP patients examination with the fluorescence technique (the examination for antinuclear factors was done by Dr Karin Stormby, Department of Bacteriology, Malmo General Hospital, Malmo) revealed a fairly high or moderate amount of antinuclear factors In 4, all women, of the 22 the thyroid antibody titer, measured by the complement fixation reaction or agglutination method was slightly increased It is known that ANF occur almost invariably in SLE ANF have also been demonstrated in Hashimoto s thyroiditis and Hijmans et al (9) have described serological overlapping between SLE and autoimmune thyroid disease It was therefore thought that the BFP patients with ANF or increased thyroid antibody titer should also be examined clinically

It has been repeatedly observed that patients with BFP may also have a circulating anticoagulant active against thromboplastin In this hospital this has been extensively studied by Laurell and Nilsson (12)

Of the 22 patients, one woman had SLE with certainty with ANF One woman had clinically suspected SLE One woman operated upon for cardiac disease and who had a previously well controlled pernicious anemia developed splenomegaly, hemolytic anemia, slight leukopenia and incrasing ESR Examination now revealed a positive ANF, increased thyroid antibody titer and slight hypergammaglobulinemia (1 5 g/100 ml) Later she died and post mortem examination revealed splenic sarcoma with metastases but no signs of collagenosis One man had chronic bronchitis and hypogammaglobulinemia Three women had slight enlargement of the thyroid and ANF, but were otherwise apparently healthy, no biopsies were performed Two women had ANF and an increased thyroid antibody titer, but were otherwise healthy One woman had ANF but was otherwise apparently healthy One woman, who at the beginning of the observation period had

TABLE III Serum abnormalities in patients with chronic BFP reaction

	♂	♀
ANF pos	1	9
Thyroid antibodies detectable	0	4
AST > 300 U	0	5
ASTA > 3 U	0	3
Bunnell pos	0	1
Serologic tests for RA	0	1
LE cells pos	0	1

TABLE IV Clinical manifestations of certain or suspected autoimmunity in 39 cases with chronic BFP reaction

	♂	♀
SLE	0	2
SLE?	0	1
Hemolytic anemia	0	1
Thyroid disorder	0	1
Rheumatoid arthritis	0	1

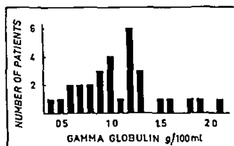


Fig 1 Gamma globulin concentration in 30 patients with chronic BFP reaction

known rheumatoid arthritis had a moderately increased thyroid antibody titer One man had ANF hypergammaglobulinemia of unknown origin and was apparently healthy Finally some patients had a slightly increased AST-titer The remaining patients of this group of 22 patients appeared healthy The coagulation studies revealed no abnormalities

Table III summarizes the serum abnormalities in chronic BFP patients A list of the clinical manifestations of certain or suspected autoimmunity seen in all 39 BFP reactors is given in table IV The distribution of the gamma globulin concentration in 30 chronic BFP patients is shown in fig 1, from which it is seen that most of the values were within the normal range of variation

Discussion

In this series of 39 chronic BFP reactors the frequency of positive ANF was conspicuously high In some of the patients the thyroid antibody titer was slightly increased *The predominance of* females was striking, the series including only one man with positive ANF and no man with an increased thyroid antibody titer

On the other hand, TPI positive patients with positive Wassermann reaction do not appear to have ANF, for no such factors could be demonstrated in a control series of 22 consecutive TPI positive and Wassermann positive patients examined at the Department of Bacteriology Malmo General Hospital

Of the 39 reactors one woman had SLE with certainty at the beginning of the observation period and in the course of the observation period SLE developed with certainty in one woman and probably in another The frequency of SLE in our BFP series 7 7 % must

therefore be regarded as relatively high In Harvey's series, where the observation period was longer, the frequency of SLE was 7 3 %

The high frequency of ANF in our series leads one's thoughts to collagen disease in which ANF are often demonstrable It is, however, noteworthy that several of our BFP reactors with ANF or increased thyroid antibody titer appeared clinically healthy Observation of these patients for a further number of years will be of great interest

Summary

A false positive Wassermann reaction may be acute or chronic Of 39 chronic biologic false positive reactors, one woman was found to have SLE at the beginning of the observation period During the observation period which ranged from 1 to 14 years (average 5 years) SLE developed in one woman with certainty and probably also in another Of 22 chronic BFP patients examined by us 9 had antinuclear factors and 4 had a slightly increased thyroid antibody titer Several of the BFP-patients were apparently healthy This relatively high incidence of SLE in our BFP series suggests that all chronic biologic false positive reactors should be followed up further All such reactors should be examined for antinuclear factors and thyroid antibodies Latent SLE should be assumed and treatment with drugs should be avoided as far as possible Vigilance is necessary during blood transfusions and pregnancy

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From the Medical Clinic (Head E Ask Upmark M D) University Hospital Uppsala Sweden

Use of Anticonvulsive Drugs in the Treatment of Recurrent Cardiac Arrhythmias

By

ANDERS PARROW

Paroxysmal cardiac arrhythmias especially tachycardia, are common and often encountered by internists as acute emergency cases The paroxysmal tachycardia constitutes a serious disadvantage as the cardiac performance during the attack in many cases is decreased to such a degree that the patient becomes unable to perform even slight work Many times, however, the attacks are of so short a duration that the patients do not have time to see a doctor before restoration to normal rhythm Prolonged attacks are of course severely incapacitating but even short ones become a heavy burden for the patient when occurring frequently Many patients experience a strong feeling of insecurity and the permanent threat of new attacks sometimes leads to considerable invalidity

The treatment of paroxysmal tachycardia has for a long time been directed mainly towards the acute attack Many drugs have been shown to be effective e g digitalis quinidine antihistamines, vasopressor substances procaine amide (4, 13, 18 20) During the last few years, several authors have reported the value of electric countershock Thus the treatment of the acute attack is no more a great problem, but for patients with frequent attacks this is not enough What they need is a therapy to prevent their attacks to enable them to lead normal lives For many years we have known that digitalis and/or quinidine, taken regularly have a good prophylactic effect on paroxysmal tachycardia, especially supraventricular attacks and that the majority of patients manage very well on such a regime

In some patients, however these remedies are not enough and for a long time, new and potent drugs have been sought to prevent recurrent tachycardia

The effect of anticonvulsive drugs on the heart was shown in 1942 by Finkelman and Arieff (10) who noted that diphenylhydantoin administration resulted in electrocardiographic changes Harris and Kokernot (12) showed that

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diphenylhydantoin given to dogs with experimentally produced myocardial infarctions, eliminated the ventricular extrasystoles The clinical application of these experiments was first made by Leonard (14) in 1958 when he gave diphenylhydantoin to a severely ill patient with ventricular tachycardia caused by a myocardial infarction In this case diphenylhydantoin seemed to be most effective

In recent years some more clinical reports have appeared (9, 19) Conn (8) administered diphenylhydantoin intra venously to 24 patients with a variety of cardiac arrhythmias and found a very good effect on the acute attack in several patients Bernstein et al (6) gave the same drug orally to prevent attacks in patients with recurrent cardiac arrhythmias and they reported reliable effects in most patients

Since 1956, anticonvulsive drugs, me phenetoin (Mesantoin®, Ciba) and di phenylhydantoin (Difhydan®, Leo), have been used in our clinic in the treat ment of cardiac arrhythmias of various kinds, mostly to prevent recurrent attacks The results have been very encouraging The discussion about the origin of cardiac arrhythmias as well as the discussion about the mechanism of action of anticonvulsives on the arrhythmias motivates a more detailed presentation of some cases

Material

All patients with some form of paroxysmal tachycardia have been interviewed in detail about the history of the illness and a routine investigation including ECG and in many cases EEG was performed Only those with more frequent and/or long lasting attacks have been treated As a rule the therapy then given has been digitalis quinidine and procaineamide With these drugs alone or in combination the majority of the patients have improved to such a degree that further therapy has been unnecessary Some however, have not improved and they have then been submitted to anticonvulsive drug therapy During the last 10 years 15 patients have been treated with mephenetoin or diphenylhydantoin (cases 1—3)

Eight patients with paroxysmal tachycardia have been treated during periods of varying length (from 4 months to 2 years) with diphenylhydantoin in doses of 0 1 g × 2—3 All of them had frequent attacks but in most cases it was not possible to get an ECG recording during the attacks Five pa tients became completely free from attacks but in one an allergic skin rash and Quincke edema made the therapy impossible Two patients were considerably improved but not quite free from attacks and on one patient this treatment had no effect at all In this case however mephenetoin 0 1 g × 2 re lieved him of his attacks

Two patients with paroxysmal atrial fibrillation have been treated One of them was completely symptom free on diphenyl hydantoin 0 1 g twice a day, the other treat ed with mephenetoin in a small dose — 0 05 g twice a day — was improved to such an extent that she did not want to increase the dose

On one patient with ventricular tachy cardia and a WPW syndrome, mephenetoin had no prophylactic effect at all Almost all these patients had previously tried con ventional therapy, digitalis and/or quinidine without any result

Apart from these patients with paroxysmal arrhythmias one patient (case 4) with continuous sinus tachycardia was also treat ed with mephenetoin

Case reports

Case 1

Unmarried woman born in 1890 No known heredity of migraine or paroxysmal tachy

cardia Her attacks of tachycardia started about the age of 45 but during the first years she did not seek medical advice At the age of 60 her attacks became more frequent and lasted longer and she was treated several times at the Medical Clinic The attacks were always of the same type an atrial tachycardia with a heart rate of of about 180—200/min Between the attacks the clinical condition of the heart and the ECGs were normal She had a slight arterial hypertension and a moderate increase in heart volume The attacks were usually stopped by intravenous injections of acetylcholine and she was given digitalis quinidine and procaineamide as continuous medication but without much benefit In the middle of the 1950s her attacks became more frequent and as a rule so prolonged that she had to visit the outpatient department 20—30 times a year Her EEG showed paroxysmal activity and in Dec 1955 she was given 0 1 g mephenetoin tablets twice a day This dose had no effect however and the patient continued to have her attacks about every second week In May 1957 the mephenetoin dose was increased to 0 1 g 4 times a day and after that she was free from symptoms. One year later her next attack occurred but this time it lasted only for 5 min and then stopped spontaneously After about 2 years of complete absence of attacks she decreased her mephenetoin dose and she had two more attacks of tachycardia The dose was increased again and during the next year she was completely free from symptoms In April 1961 she had an operation for a fracture of the femoral neck. During her stay at the Surgical Clinic she was not given mephenetoin and after 2 weeks her tachycardia reappeared Two weeks later she suddenly died Post mortem examination showed the cause of death to be a pulmonary embolus In the heart was only found slight atheromatosis of the coronary arteries and a moderate hypertrophy

Summary An elderly woman presenting 20 years history of extremely frequent and prolonged attacks of supraventricular tachycardia, without any signs of underlying cardiac disease but with a paroxysmal EEG No earlier treatment had prevented her attacks, but during four years' therapy with mephenetoin she was almost completely free from attacks

Case 2

An unmarried woman born in 1935 without known family history of epilepsy migraine or paroxysmal tachycardia came to the outpatient department in June 1957 complaining of attacks of tachycardia The attacks started and ended abruptly and were only of some minutes duration but in spite of this they frightened her a great deal Routine examination between the attacks including an ECG did not show any abnormalities and her attacks were interpreted as paroxysmal tachycardia Though they occurred seldom but with the good results of case 1 in mind we gave her mephenetoin in a small dose 0 1 g twice a day Thereafter she was free from tachycardia but some months later she experienced attacks of unconsciousness which made the diagnosis of epilepsy likely An EEG was taken and during the recording she developed a typical grand mal attack A careful neurological examination was performed including encephalography but no organic disease causing her grand mal could be found Her mephenetoin dose was increased to 0 1 g three times a day and since then the patient has been free from symptoms for 8 years

Summary A young woman with attacks of paroxysmal tachycardia which were possible to prevent by mephenetoin medication developed a classical grand mal attack six months later

Case 3

Man born in 1922 without known familial background of migraine or paroxysmal tachycardia and previously mainly in good

in the EEG are in fact a not uncommon phenomenon in cases of paroxysmal arrhythmias (own unpublished observations) The remarkably good effect of anticonvulsive drugs in preventing as well as stopping acute attacks of paroxysmal tachycardia and other paroxysmal arrhythmias, may not, however, be taken as further evidence of the epileptic origin of these cases Certainly the anti convulsive drugs act upon the CNS, and it seems reasonable to presume that their effect on the cardiac arrhyth mias is at least partly due to the CNS action But, as shown by Harris et al (12) and Leonard (14), the drugs al o exert an effect on cardiac arrhythmias, which are definitely released from the heart itself, which also makes a direct action on the heart likely

Whatever the etiology of paroxysmal tachycardia and whatever the mechan ism of anticonvulsive drugs may be, they are to be recommended in the treatment of recurrent arrhythmias of different kinds, especially in those cases where the ordinary cardiac drugs are ineffective It is also worth mentioning that the different anticonvulsive drugs are not of the same efficiency in all individuals If for instance no result is obtained with one anticonvulsive drug another should be tried

Summary

During the last 10 years 15 patients with arrhythmias uncontrolled by the usual cardiac drugs, digitalis and/or quinidine have been treated with anti convulsive drugs (mephenetoin-Mesantoin® — and diphenylhydantoin Difhydan®) One patient had a paroxysmal ventricular tachycardia 2 had paroxys mal atrial fibrillation, one a continuous sinus tachycardia of one years duration Two patients had paroxysmal atrial tachycardia and in 8 the attacks were never recorded on ECG and could not therefore be classified None of these patients had any detectable underlying cardiac disease Eleven were completely free from attacks during treatment with anticonvulsive drugs and 3 improved In one patient with a WPW syndrome and attacks of ventricular tachycardia therapy had no effect

Some patients had pathological EEG and one, in fact, developed a grand mal epilepsy

The etiology of paroxysmal tachycardia and the relation to migraine and epilepsy are discussed In spite of the fact that the mechanism of action of anticonvulsive drugs is not fully known, it is concluded that these drugs are of value in the prevention of recurrent cardiac arrhythmias of different kinds

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From the Clinic of Pulmonary Diseases University of Helsinki and the Laakso Hospital
Helsinki Finland

The Effect of Bronchodilator Aerosols on the Peak Expiratory Flow Rate in Asthmatic Patients

By

Mauri Mattila and Antero Muittari

Both adrenergic α and β receptors are believed to exist in the mammalian tracheo bronchial tree Stimulation of β receptors leads to the relaxation of the bronchial smooth muscle, whereas the increase in tone of the smooth muscle caused by sympathomimetics is mediated by α receptors (1, 2) The β effect pre dominates and the contracting α influence is not to be found in all animal species, e g Foster (6) was able to demonstrate only β receptors in the isolated tracheal chain of the guinea pig Isoprenaline and related compounds which affect mainly β receptors are among the most effective bronchodilator drugs *Likewise the specific blockade of* β receptors e g by propranolol, has aggravated the condition of asthmatic patients (8 9) The effect of orciprenaline was found to be of longer duration in asthmatics than that of isoprenaline (12 13)

Several bronchodilator combined prep arations have been marketed, which in addition to isoprenaline contain either atropine methonitrate, or phenylephrine, a vasoconstrictor sympathomimetic drug These preparations have been described as being superior to isoprenaline alone The purpose of this present study is to compare the clinical efficacy of some combined preparations with that of isoprenaline or orciprenaline alone

Material and methods

The patients were 12 asthmatics the criter ion of asthma being the definition suggested by the Ciba Symposium (4) an intermit tent or reversible generalised airway ob struction

Most of the patients were hospital in patients and were being treated with corti *costeroids A few were outpatients coming* from home for investigation On the morning of the test patients did not receive any drugs The aerosols and doses used were iso- prenaline sulphate 0 2 mg (Medihaler Iso® Riker) isoprenaline sulphate 0 2 mg + atro- pine methonitrate 0 08 mg (Medihaler Bron® Riker) isoprenaline sulphate 0 2 mg + phenylephrine hydrochloride 0 3 mg (Medihaler Duo® Riker) orciprenaline sulphate 0 7 mg (Alupent® Boehringer) and isoetharine methane sulphonate 0 35

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TABLE I Absolute (l/min) and per cent improvement of the peak expiratory flow values (mean ± S E M)

Aerosol	5 min	15	30	60	120
Placebo (12)	29±8 10±3%	16±10 8±3%	7±18 8±7%	24±22 13±9%	10±25 7±6%
Isoprenaline Medihaler Iso (12)	109±17 50±9%	92±14 43±7%	75±15 35±8%	45±16 25±8%	27±13 13±6%
Isoprenaline+atropine methonitrate Medihaler Bron (11)	91±12 48+13%	89±17 40±9%	67±14 28±5%	69±15 29±9 %	49±14 23±5%
Isoprenaline+phenylephrine Medihaler Duo (12)	112±16 57±10%	94±16 45±9%	70±14 35±8%	63±13 33±8%	21±17 14±8%
Orciprenaline Alupent (11)	73±14 31±6%	87±17 36±7%	93±15 41±8%	92±16 40±7% $P<0\ 05$	72±15 34±8% $P<0\ 05$
Isoetharine+phenylephrine +thenyldiamine Bronchilator (11)	82±11 46±9%	97±16 19±12%	86±15 41±8%	81±17 36±9% $P<0\ 05$	57±17 25±9%
Healthy controls without aerosol (9)	1 8±0 1%	0 3±1 0%	1 4±0 5%	−0 8±1 1%	0 2±0 9%

mg + 0 35 phenylephrine hydrochloride 0 07 mg − thenyldiamine hydrochloride 0 03 mg (Bronchilator® Winthrop) and the placebo spray (Riker) It was considered essential for each experiment that the peak expiratory flow (PEF) rate of the patient was about 50 % of its maximum value since it has been found (7) that the bronchodilator effect of isoprenaline was the most favourable at this degree of expiratory impairment If the condition of the patients was such that PEF was much higher or much lower than 50 % the test was not carried out

The patients had been taught in advance to master a proper expiratory technique and the use of the Wright s Peak Flow meter At first the pulse rate was measured in a sitting position then 5 consecutive PEF determinations were made in the same position The mean of the 2 best expirations was taken as the PEF value After that the patient was allowed to inhale spray of one of the aerosols, two inhalation doses of Medihaler Iso were needed to provide the same dose of isoprenaline as other isoprenaline preparations gave in one dose The recording of the pulse and PEF were repeated in a sitting position at 5 15 30 60 and 120 min after the spray It was impracticable to carry out a double blind trial but the patients did not know what drug they received When receiving placebo they were told that this spray contained a new bronchodilator drug

Every patient was tested with at least five of the aerosols the placebo and at least four of the other aerosols One aerosol only was tested on any one day on one patient (see table I)

Control investigations were made on nine nurses in normal health who after mastering the standard expiratory technique were subjected to a PEF test without aerosols at the same intervals as in the tests on patients Such control investigations were not made on patients because they would have been unable to co-operate without a suitable aerosol

In the statistical treatment of the results Student s t test was used

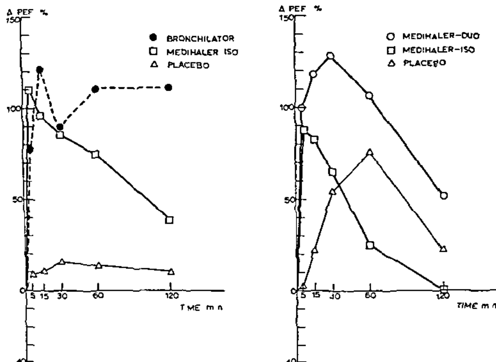


Fig 1 On the left is a normally reacting asthmatic on the right a "placebo-reactor

Results

Both the absolute (l/min) and the per cent PEF changes are given in table I The PEF values of healthy subjects were unaltered throughout the two-hour period In patients placebo caused a 10 per cent average improvement of the PEF at 5 min ($p < 0.01$) Although the mean per cent improvement from the starting values at 60 min was even larger it was no longer statistically significant All bronchodilator aerosols differed significantly from placebo at 5 15 and 30 min without differing significantly from each other At 60 min only Alupent and Bronchilator differed significantly from placebo ($p < 0.05$) and at 2 hours only Alupent differed statistically from placebo ($p < 0.05$) Analysis of the effect of placebo in individual cases disclosed that in 3 patients the placebo-induced PEF improvement was markedly high In one patient the placebo reached its peak effect at 60 min and in two patients within two hours Fig 1 presents test results of one placebo reactor and of one placebo non reactor The favorable effect of a bronchodilator aerosol is also to be seen in "placebo reactors because PEF improves after such an aerosol much more rapidly Fig 2 illustrates test results for two other patients one of whom was a placebo reactor It emerges from this figure that in a placebo non reactor Mediha ler Duo was still extremely active at

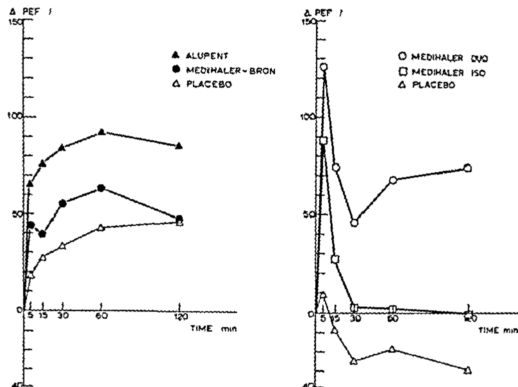


Fig 2 On the left is one of three placebo reactors On the right a normal asthma patient In this case the effect of Medihaler Duo is much better than that of Medihaler Iso

120 min although its mean values did not differ significantly from placebo Even in placebo reactors the subjective feeling of relief after placebo was generally weaker than that after the bronchodilator aerosol, probably being due to the more rapid change in PEF after the medicinal spray None of the aerosols caused tachycardia or other harmful side effects As a rule the pulse rate decreased after the inhalation having been abnormally high at the outset due to the airways obstruction

Discussion

The advantage of β sympathomimetics as bonchodilator drugs is that they directly affect the bronchial smooth muscle relaxing its spasm irrespective of its etiology The particular usefulness of isoprenaline is the rapid onset of its effect Unfortunately the effect also disappears quickly, and an overdosage of isoprenaline is quite common in the treatment of severe asthmatic attacks Since tachycardia is an exceedingly distressing side effect of overdosage, attempts to introduce longer acting isoprenaline congeners such as isoetharine and orciprenaline are welcome Other attempts have been made to achieve a longer effect by combining atropine methonitrate or phenylephrine with isoprenaline As an anticholinergic drug the former prevents secretion of mucus while the latter relieves the swelling

of the mucous membrane Isoprenaline if used alone, may cause the mucous membrane to swell still more by vaso dilation

A number of comparative studies on long acting broncholytics have been carried out and the results are at least partly controversial Such investigations are however, beset with considerable difficulties One such difficulty is the spontaneous 24 hour variation in the respiratory function of asthmatic patients Scherrer et al (10) observed the airway resistance of asthmatic patients between 8 a m and 6 p m and they found that the airway resistance was lowest at 12 noon, while markedly higher values were measured in the morning and in the evening It would be useful to determine the patient s 24 hour rhythm before studies with bronchodila tor drugs The considerable placebo effect found in this study was at least partly due to the fact that most experi ments were made between 9 a m and 11 a m and within these two hours the patients condition had already spon taneously improved The mean place bo effect in this study was further en hanced by the existence of 3 real placebo reactors , among the group of 12 patients In several earlier broncho lytic experiments placebo had not been used at all or the placebo effect had been observed for 15 min only

It has been disputed as to which is the most suitable respiratory function test when the effects of bronchodilators are being evaluated The $FEV_{1.0}$ determina tion has been generally regarded as a reliable method Cohen (5) used in his comparative studies on bronchodilator aerosols, McKesson s Vitalor spirometer and he considered MEFR (maximal expiratory flow rate) clearly better than $FEV_{1.0}$ of FVC (forced vital capacity) as a standard for changes in the respira tory function On the other hand 3 consecutive FVC determinations can be a fairly considerable exertion for the patient and may provoke bronchospasm Determination of PEF by Wright's Peak Flow meter represents a much smaller exertion for the patient In pharmacological experiments particu larly PEF has been proved to be suffi ciently sensitive and reliable (11) In all the foregoing methods the patient must co-operate completely The deter mination of the airways resistance for example by the body plethysmograph is by far the best method, because it is very sensitive and does not require any considerable co-operation from the patient

Many authors consider the combina tion of isoprenaline + atropine metho nitrate more effective than isoprenaline alone For instance Chamberlain et al (3) found the effect of this combination to be still near its maximum after three hours, whereas the effect of isoprenaline had already fallen to below half of its maximum after one hour The reason why we were not able to confirm the advantage of that combination probably was that our patients were asthmatics in a reasonable dry stage in whom the bronchial secretion and the swelling of the mucous membranes were mini mal It is likely that atropine methoni trate was unable to contribute any addi tional effect under these circumstances Possibly for the same reason the combina

tion phenylnephrine + isoprenaline was not more beneficial than isoprenaline in our tests, although some excellent results have been reported with it (5) Combinations of drugs perhaps are relatively more effective in spastic chronic bronchitis than in "pure' asthma The beneficial effect of the isoetharine + phenylephrine + thenyldiamine in this study probably was based mainly on the effect of isoetharine because the combined preparation isoprenaline + phenylephrine was not as effective as the former combination at one hour after the administration, and because antihistamine drugs are not considered essential in the treatment of asthma

Since, however different patients reacted in an individual manner to the same aerosol, it is reasonable to try on every asthmatic patient e g three different preparations, and choose the one producing the greatest rise in PEF at one hour after the inhalation of the drug

Summary

Twelve asthmatic patients have been examined for the effect of 5 different bronchodilator aerosols and placebo on the peak expiratory flow rate (PEF) The aerosols studied and the quantities of substances contained in one dose were isoprenaline sulphate O 2 mg (Medihaler—Iso®, Riker), isoprenaline sulphate 0 2 mg + atropine methonitrate 0 08 mg (Medihaler Bron® Riker), isoprenaline sulphate 0 2 mg + phenylephrine hydrochloride 0 3 mg (Medihaler-Duo®, Riker) orciprenaline sulphate 0 7 mg (Alupent®, Boehringer), isoetharine methane sulphonate 0 35 mg + 0 35 phenylephrine hydrochloride 0 07 mg + thenyldiamine hydrochloride 0 03 mg (Bronchilator®, Winthrop), and the placebo aerosol (Riker) The respiratory function was studied with Wright's Peak Flow meter, PEF was measured at the start and after the lapse of 5 15, 30, 60 and 120 min since administration Among the series of 12 patients there were 3 placebo reactors' After 30 min all aerosols were better than placebo, but they did not differ significantly from each other After one hour only Alupent® and Bronchilator® differed significantly from the placebo, and after the lapse of 2 hours only Alupent® was significantly better than placebo

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From the National Health Service of Denmark Statistical Section Copenhagen Denmark

Coronary Occlusion in Denmark

Morbidity and Mortality

By

JOHANNES MOSBECH and KAREN DREYER

The mortality rate from arteriosclerotic heart disease has been steadily increasing for some years in Denmark (fig 1) (5) as well as in many other countries (2 6, 14), and it is the general impression that the acute manifestation of the disease — the coronary occlusion — also occurs with increasing frequency

In order to get more exact national figures admissions to hospitals for acute coronary occlusion were made notifiable in Denmark (4 7 mill inh) during a two month period The National Health Service asked hospital departments all over the country where patients with coronary occlusion are admitted, i e departments of internal medicine and unspecialized hospitals in the provincial districts, to report cases of coronary occlusion admitted during the months of March and April 1963 The present paper presents the results of this country wide study combined with an analysis of deaths certificates from the same period

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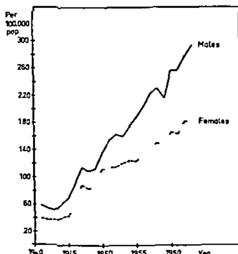


Fig 1 Mortality rates from arteriosclerotic heart diseases per 100 000 population by sex Denmark 1941—1963

Hospital material

The form used for reporting contained in addition to questions about the patient s name age sex date of birth occupation residence and length of stay in hospital a few clinical questions thus allowing for an

TABLE I Hospitalized cases of coronary occlusion classified by sex age and whether discharged or died

	♂			♀		
Age (yrs)	Discharged alive	Deaths	Total	Discharged alive	Deaths	Total
30 –	1	1	2	–	1	1
35 –	4	1	5	2	–	2
40 –	14	5	19	2	–	2
45 –	28	4	32	6	1	7
50 –	70	10	80	14	3	17
55 –	78	35	113	15	11	26
60 –	75	42	117	18	9	27
65 –	81	57	138	44	30	74
70 –	57	47	104	35	36	71
75 –	45	48	93	23	36	59
80+	21	33	54	13	38	51
Total	474	283	757	172	165	337
Average age (yrs)	62 5	68 0	64 6	66 8	72 4	69 6

TABLE II Estimated annual number of hospitalized cases of coronary occlusion by age and sex

	Estimated no per year		Per 100,000 pop per year	
Age (yrs)	♂	♀	♂	♀
30 –	42	18	14	6
40 –	306	54	100	17
50 –	1 158	258	423	90
60 –	1 530	606	791	278
70 –	1 182	780	1 089	613
80+	324	306	942	736
Total	4 542	2 022	196	86
Age 30 years and over			373	156

evaluation of the validity of the diagnosis It was asked whether the patient had had angina pectoris or coronary occlusion earlier and whether the clinical findings ECG and transaminase values were typical

The notification forms were sent to 138 departments which all willingly co-operated in the study

The total number of reported cases of acute coronary occlusion was 1 094 evenly

distributed over the two months with about 18 cases per day The age and sex distribution according to outcome (discharge or death) is shown in table I The male/female ratio was 2 25 1 e 69 % were males The sex ratio showed a decrease from 5 7 at the age 40—49 years to 1 1 for patients aged 80 years and over The average age for surviving males was 62 5 years and for lethal cases 68 0 years whereas the female patients were on an average almost 5 years older in both groups

On the assumption that the two months March and April are representative for the whole year the estimated annual number and rates per 100 000 for hospitalized cases have been computed in table II

The rates increase with increasing age for both sexes but there is a striking discrepancy between the sexes the rates for males in the age group 70—79 years being about 2 1/2 times that for the age group 50—59 years whereas the corresponding ratio for females is almost 7 1 As coronary occlusion very seldom occurs in the ages under 30 years the rates per 100 000 for the population aged 30 years and over are shown at the bottom of the table

The crude lethality rate for this material of hospitalized cases of coronary occlusion was 41 0 % namely 37 4 % for males and 49 0 % for females (table III) The rate for males increased from about 20 % at the age of 40 years to a maximum of 60 % for patients over 80 years whereas the rate for females increased from 33 % at the age of 50 years to 75 % at the age of 80 years and over It appears from the table that the higher crude lethality rate for women is not due to the different age-distribution for the two sexes

The distribution as to length of stay in hospital by sex and outcome (discharge or death) is shown in table IV It should be noted that in most cases the date but not the hour of admission and discharge or death was known and for this reason it was not possible to obtain an exact distribution according to length of stay Some of the patients who died on the date following

TABLE III Lethality in per cent of hospitalized cases of coronary occlusion by age and sex

Age (yrs)	Per cent lethality	
	♂	♀
30—	28 6	—
40—	17 6	—
50—	23 3	32 6
60—	38 8	38 6
70—	48.2	55 4
80+	61 1	74 5
Total	37 4	49 0

admission but really within 24 hours are thus included in the group 1 day in table IV

The majority of the surviving patients namely 52 % remained in hospital for 4—6 weeks an additional 30 % were hospitalized for 2—4 weeks and 3 % were discharged within the first two weeks often against medical advice whereas 15 % of the surviving patients had to stay in hospital for more than 6 weeks

Of the fatal cases 36 % died on the day of admission or the following day another 30 % within the rest of the first week 18 % in the second week and 16 % later than two weeks after admission

The total number of bed-days amounted to 25 000 1 e 150 000 bed-days during a whole year This amounts to 1 7 % of all bed-days in Danish hospitals viz 5 % in specialized departments of internal medicine and 1 4 % in non specialized hospitals

The lethality rates by sex and length of stay are shown in table V It appears that most of the difference in lethality between the sexes was due to an excess lethality in females within the first week after admission

A history of previous coronary occlusion was reported in 226 cases or in about 20 % of all patients The lethality rates for these

TABLE IV Hospitalized cases of coronary occlusion according to length of stay sex and whether discharged or died

	♂		♀		♂ and ♀	
Length of stay (see page 431)	Discharged alive	Deaths	Discharged alive	Deaths	Discharged alive	Deaths
Under 24 hours	—	72	—	26	—	98
1 day	1	35	—	29	1	64
2 days	—	21	—	15	—	36
3 days	—	9	—	10	—	19
4—7 days	4	46	2	35	6	81
8—14 days	10	55	5	24	15	80
15—28 days	148	28	44	18	192	46
29—42 days	250	12	84	3	334	15
43 days and over	61	4	37	5	98	9
Total	474	283	172	165	646	448
Total no of bed-days	15 631	2 196	6 117	1 348	21 748	3 544
Average length of stay (days)	33 0	7 8	35 6	8 2	33 7	7 9

TABLE V Lethality rates by sex and length of stay

Time of death in relation to admission	Lethality in %	
	♂	♀
1st and 2nd day	14 1	16 3
Rest of 1st week	10 1	17 9
2nd week	7 4	7 1
3rd week or later	5 8	7 7
Total	37 4	49 0

patients were slightly higher than for other patients but the figures are too small to allow for any definite conclusions The reported number of cases of previous attacks is probably underestimated, as a complete medical history has been difficult to obtain in the cases where the patient died shortly after admission

Tables VI and VII include a summary of the clinical information given in the individual reports Information about a history of agina pectoris was given for about one half of the patients, and the present clinical findings were typical in about 90 % of the cases The ECG picture was typical for coronary occlusion in about 80 % of the cases and the GOT values were typical in 76 % of the surviving patients but were examined in only 62 % of the fatal cases They were typical in 85 % of the patients examined

As can be seen from these figures and from the frequency of affirmative answers shown in table VII, the diagnosis of coronary occlusion may be considered reasonably well established in this material

TABLE VI Percentage distribution of answers to questions about validity of diagnoses

Question	Discharged alive (%)			Deaths (%)		
	Yes	No	Not known	Yes	No	Not known
1 Previous coronary thrombosis	18	81	1	24	73	3
2 Angina pectoris	54	45	1	53	42	5
3 Clinical findings typical	93	7	—	86	12	2
4 ECG typical	85	15	—	72	18	10
5 GOT typical	76	18	6	53	9	38

TABLE VII Affirmative answers to questions 2—5 (see table VI)

	Total number		Per cent	
	Discharged alive	Deaths	Discharged alive	Deaths
Affirmative answers to all 4 questions	211	105	33	24
Affirmative answers to 3 questions	295	175	46	39
Affirmative answers to 2 questions	117	95	18	21
Affirmative answer to 1 question	22	48	3	11
Negative answers or unknown to questions 2—5	1	24	0	5
Total	646	448	100	100

Death certificate material

An analysis of death certificates from the months March and April 1963 has been carried out comprising all deaths from coronary occlusion except those already included in the hospital material

It was found reasonable to divide this material into two groups according to the degree of reliability of diagnosis Thus *group A* comprised cases of coronary occlusion stated to have lasted more than half an hour cases of sudden death ascribed to coronary occlusion (where the diagnosis was supported by information about other manifestations of arteriosclerotic heart disease such as angina pectoris previous coronary occlusion etc) and finally cases of sudden death without such information but where the diagnosis of coronary occlusion was confirmed by autopsy *Group B* on the other hand comprised cases in which the patient died instantaneously and in which no corroborative information as to the diagnosis was available A detailed account of the sub-groups constituting groups A and B is shown in table VIII separately for each sex

Group A comprised 79 % of the total material of 1 130 deaths namely 549 males out of a total of 700 and 340 females out of a total of 430 whereas 21 % of the deaths belonged to group B sudden death ascribed to coronary occlusion without further verification

TABLE VIII Cases of coronary occl is on known from death cert ficates only grouped accord ng to val d ty of d agnosis and by sex

		Total number		Per cent	
		♂	♀	♂	♀
Group A					
I	Durat on more than 30 m n or instantaneous death w h d agnos s confirmed by autopsy	322	222	46 0	51 6
II	Instantaneous death w th ment on of previous coronary occlus on	50	15	7 1	3 5
III	Instantaneous death w th ment on of ang na pectoris	39	15	5 6	3 5
IV	Instantaneous death with ment on of other s gns of arter osclerot c heart d sease	138	88	19 7	20 5
Group B					
V	Instantaneous death w th ment on of other form of heart d sease	44	22	6 3	5 1
VI	Instantaneous deatl v thout ment on of other heart d sease	107	68	15 3	15 8
Total		700	430	100 0	100 0

TABLE IX Cases of coronary occlus on known from death cert ficates only by age and sex (con cern ng defin t on of groups A and B see table VIII)

	♂			♀		
Age (yrs)	Group A	Group B	Total	Group A	Group B	Total
30–	2	1	3	–	1	1
40–	22	10	32	1	1	2
50–	62	25	87	15	9	24
60	163	47	210	81	18	99
70–	176	38	214	143	28	171
80+	124	30	154	100	33	133
Total	549	151	700	340	90	430
Average age (yrs)	70 5	67 8	69 9	74 6	73 6	74 4

The total number of deaths aris ng from coronary occlusion during the months March and April 1963 and not included in the hospital material amounted as mentioned above to 1 130 evenly d str buted on the two months with 18—19 deaths per day The age and sex d str bution is sl own in table IX separately for groups A and B Almost

62 % were males as against 69 % in the hospital material It appears from the table that group A included relatively more old people than did group B namely 55 % males over 70 years in group A as against 45 % in group B The corresponding figures for females were 71 % as against 68 % The sex ratio was the same in the two groups M F = 1 63 The average age was about 70 years for males and 75 years for females i e the same age difference of about 5 years as found in the hospital material

In tables X—XII the two materials have been combined in order to estimate the total annual morbidity and mortality from coronary occlusion All computations were carried out both with and without the more unreliable group B but those deaths within the hospital material which occurred after the end of April 1963 were excluded (altogether 48 deaths)

Table X shows the 1 530 deaths reported during the two months by age and sex Out of these 678 or 44 % occurred in hospital namely 400 from the hospital material and 278 deaths not reported by the hospitals either because the patients had been admitted before 1 March or because the patients died in hospital departments to which they were admitted because of other diseases e g surgery (these departments being for practical reasons not included in the survey)

A comparison with the official figures for deaths from coronary occlusion must be based on the figures including group B as doubtful cases of coronary occlusion are coded to this cause of death in the statistics Based on the figures in table X the annual number of deaths from coronary occlusion in Denmark can be estimated as altogether 9 200 namely 5 700 males and 3 500 females According to Causes of death in Denmark the number of deaths from coronary occlusion in 1963 amounted to 5 632 males and 3 144 females It seems thus as if the figures obtained for March and April give a slight overestimation of the mortality for females

The estimated annual mortality rates per 100 000 are shown in table XI by age and sex The male mortality rate is 60—70 % higher than the female one

Based on the combined material the annual rates for incidence of coronary occlusion have been computed by age and

TABLE X Total number of deaths from coronary occlusion reported during two months by age and sex (concerning definition of groups A and B see table VIII)

	♂				♀			
	Reported from	From death certificates			Reported from	From death certificates		
Age (yrs)	hospitals	A	B	Total	hospitals	A	B	Total
30	2	2	1	5	1	—	1	2
40—	8	22	10	40	1	1	1	3
50—	39	62	25	126	13	15	9	37
60	86	163	47	296	35	81	18	134
70	88	176	38	302	63	143	28	234
80+	31	124	30	185	33	100	33	166
Total	254	549	151	954	146	340	90	576
Herein hospitalized	254	143	15	412	146	110	10	266

TABLE XI Estimated annual death rates per 100 000 from coronary occlusion by age and sex (concerning definition of groups A and B, see table VIII)

	♂		♀	
Age (yrs)	Group B excluded	Group B included	Group B excluded	Group B included
30—	8	10	2	4
40—	59	78	4	6
50—	221	276	59	77
60—	773	918	319	369
70—	1 459	1 669	972	1 104
80+	2 705	3 228	1 922	2 398
Total	208	247	124	147
Total over 30 years	396	470	226	267

TABLE XII Estimated annual incidence per 100 000 of coronary occlusion, by age and sex (concerning definition of groups A and B, see table VIII)

	♂		♀	
Age (yrs)	Group B excluded	Group B included	Group B excluded	Group B included
30—	18	20	6	8
40—	141	160	19	21
50—	546	600	119	138
60—	1 257	1 402	490	540
70—	2 023	2 233	1 245	1 377
80+	3 071	3 595	2 109	2 586
Total	331	370	168	191
Total over 30 years	630	704	305	347

sex in table XII The difference in morbidity between the sexes is greater than found for the mortality (table XI) the male morbidity being about twice that of the female This is due to a greater preponderance of males in the non fatal cases viz 73.4 % males as against 62.3 % in the fatal cases The doubtful cases in group B give rise to some uncertainty in the estimation of morbidity as well as of mortality from coronary occlusion Thus the morbidity rates are 12—14 % higher and the mortality rates about 18 % higher if this group is included

Discussion

With the aim of investigating the epidemiology of coronary heart disease in general figures on mortality and

morbidity have been reviewed in a number of countries (6)

Mortality from arteriosclerotic heart disease has shown interesting trends both nationally (1 2, 4, 5 6, 14) and in international comparisons (11), but the method has certain limitations, primarily inaccuracy of diagnosis and differing fashions in death certifications as well as varying coding practices (12)

Population studies including regular health examinations of population samples have given valuable information about the prevalence and the incidence of coronary heart disease (10, 15) Considerable variations have turned up however, partly because of the great difficulty in establishing mutually acceptable diagnostic criteria and definitions of what is understood by arteriosclerotic heart disease in spite of much work on standardization within this field in recent years For this reason it has been felt advisable to limit this study to include only acute coronary occlusion (infarction) for which generally accepted criteria exist

A number of Scandinavian investigations of the incidence of acute coronary occlusion have been undertaken recently in Oslo (16 17) 1961, Malmo (3 13) 1963 Helsinki (8) 1961, and Copenhagen (7) 1960, but no countrywide investigations concerning morbidity of acute coronary occlusion have so far been published

Westlund and Hougen (16 17) in the city of Oslo found an incidence of first attack of coronary occlusion for males of 13 per 10 000 at the age of 40—44 years, increasing to 116 at the age of 60—64 years These figures are consistently higher than the present ones for Copenhagen from the age of 45 years but no reasonable explanation for this discrepancy can be given

Sievers material included 2 904 patients admitted to hospital in the regional hospital of Malmo 1935—1959 The incidence figures for acute coronary occlusion in Malmö and Copenhagen show good correlation The average age of the patients was 64 years 62 years for males and 68 years for females The sex ratio was 1 8 males 1 female in the total material but this ratio decreased from 6 6 at the age of 30 years to 1 at the age of 70 years

The lethality within the first 4 weeks was 35 per cent viz 32 per cent for males and 38 per cent for females, as compared with our lethality rates of 39 35 and 47 per cent respectively

As regards age and sex distribution our material is in good agreement with other Danish materials of patients with coronary occlusion from hospitals (9)

The question remains how does a study of hospital admitted cases of coronary occlusion reflect the real incidence of the disease? It can be assumed that in Denmark at the time of the examination, practically all patients seriously suspected of having a coronary occlusion would be admitted to one of the hospitals included in the study Furthermore all clinical departments asked for notification reported willingly — and we have reason to believe extensively — about the patients admitted A limited number of cases may have occurred in specialized departments other than internal medicine but these have for practical reasons been left out

It is quite clear, however, that an unknown number of patients with only vague and uncharacteristic symptoms were not diagnosed and thus not hospitalized for their coronary occlusion This caused an underestimation of the disease incidence, based on analysis of hospital patients

The group of patients with coronary occlusion who die from their disease before being admitted to hospital can be corrected for by an analysis of death certificates as tried in the present study Death certificates are often insufficiently substantiated, as has been shown in several studies (12), and therefore a division of the material according to the quality of the information on the certificates has been made

The difficulties inherent in determining the real incidence of coronary occlusion are also borne out by the fact that 45 per cent of the patients who die from their first diagnosed infarction are found by autopsy to have evidence of previous infarctions This applies to patients who have hitherto been without symptoms as well as to patients who have a history of angina pectoris It must be assumed that there exists a not inconsiderable group of undiagnosed cases of coronary occlusion, which have not given any symptoms (16) It is, however, justified to assume that the group of undiagnosed cases of coronary occlusion tends to affect materials obtained under similar conditions to a similar degree

Summary

Individual reporting of all cases of acute coronary occlusion hospitalized in departments for internal medicine or in mixed medical surgical departments was carried out in Denmark during the period 1 March to 30 April 1963

The problem of fatal non hospitalized cases of coronary occlusion was dealt with by an analysis of the death certificates from the same period

The total number of hospitals and hospital departments included in the investigation was 138, and 1,094 cases of acute coronary occlusion were reported during the two months The average number of patients, admitted per day was 18, and 70 per cent were males

The average age was 64 6 years for males and 69 6 for females The average age for fatal cases was 5 1/2 years above that for survivals

The total lethality for hospitalized cases was 41 per cent, viz 37 4 per cent for males and 49 0 per cent for females The lethality rate for males increased from about 20 per cent at the age of 40 years to about 60 per cent at the age of 80 years, whereas the female lethality rate increased from 33 per cent at the age of 50 years to 75 per cent at the age of 80 years

An analysis of the fatal cases showed that 30—35 per cent of all deaths occurred on the day of admission or the following day

On the assumption that the two month period in question is representative for the whole year, a total of 1,500 male and 2,000 female hospitalized cases may be expected annually The age distribution is shown, both in actual figures and as rates per 100,000

The average hospital stay was 34 days for survivals and 8 days for fatal cases

The total number of bed-days was 25,000 corresponding to an annual number of days of 150 000

In departments for internal medicine, coronary occlusion was responsible for 5 per cent of all bed days, and in mixed medical surgical departments for 1 4 per cent The total annual number of bed days, 150,000, corresponds to 1 7 per cent of all bed-days in all general hospitals in Denmark

The clinical information concerning the validity of the diagnosis showed that 20 per cent of the hospitalized cases had had one or more previous attacks of coronary occlusion 50 per cent had previously had angina pectoris, 80 per cent showed typical electro cardiographical changes, and 90 per cent had typical clinical findings, whereas 76 per cent of the surviving patients had typical GOT figures

The death certificates for all fatal cases of acute coronary occlusion in Denmark during the months March and April 1963 have been reviewed and those already known from the study have been excluded An analysis of this material showed that 700 males and 430 females died from coronary occlusion during the two-month period in addition to the fatal cases reported from hospitals The average age for the deaths was 69 9 years for males and 74 4 years for females

In order to get an estimate of the total morbidity from coronary occlusion an analysis of the combined material of all known cases during the two month period was carried out The result was that there were 1 530 deaths from coronary occlusion during this period of which 678 or 44 per cent occurred in hospital It may thus be expected that about 9 200 deaths from coronary occlusion occur annually, of which 5,700 are males and 3,500 females

The mortality rate for males was at a level of 200—250 and that for females 125—150 per 100,000 The excess mortality of males over females was 60—70 per cent The morbidity rates were 330—370 per 100,000 and 170—190 per 100,000 respectively for the two sexes, thus the excess morbidity of males over females was almost 100 per cent

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From the Department of Neurology Hesperia Hospital Helsinki the Department of Medicine Kivela Hospital Helsinki and the Third Department of Medicine University of Helsinki Helsinki Finland

Electroencephalographic Alterations in Diabetes Mellitus

By

THEODOR JAKOBSON ERKKI HUHMAR and RANAN RIMON

Alterations of the electroencephalogram (EEG) have been reported by some authors to occur frequently in so called labile diabetics Thus Greenblatt (8) observed a pathological EEG in 18 out of 35 cases in whom the diabetic state was difficult to control and similar observations have been made by other investigators (4 13, 16)

Izzo et al (11) on the other hand could not find any correlation in a material consisting of 77 diabetics, between the alterations in the EEG on the one hand and the severity or the lability of the diabetic state on the other Andersson and Kirstein (1) more recently observed a pathological EEG in 95 (49 %) out of 194 diabetics but the frequency of alterations of the EEG in those diabetics who were characterized as labile cases was not higher than in the others The pathological findings in the EEG consisted in this series of unspecific pathological activity in 57 cases focal changes in 29 cases and paroxysmal activity in 9 cases These alterations were in most cases attributed to cerebral vascular complications although it was concluded that post hypoglycemic brain damage in some cases also could be involved in the production of the electrocerebral derangements

Because of these somewhat divergent reports it was thought worthwhile to re investigate the occurrence of EEG alterations in diabetic patients A material consisting of 40 diabetics in whom EEG tracings had been obtained was analyzed with respect to the frequency and severity of hypoglycemic episodes and the occurrence of keto-acidosis In addition the known duration of diabetes the insulin requirement and the degree of vascular lesions were estimated and an attempt was made to correlate these clinical findings with the EEG changes observed in the same patients

Material and methods

The material consists of 40 diabetic patients 18 males and 22 females who were hos-

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pitalized in the neuropsychiatric wards of the Hesperia Hospital the Medical Department of the Kivela Hospital or the Third Medical Department of Helsinki University

The known incidence of diabetes in the relatives of the patients included in the study was 37 % (15 cases), and 2 relatives of the patients were known to have epilepsy The average age of the patients was 43 6 years (range 16 to 71 years) and the known duration of the disease 9 6 years (range 1 to 27 years) Altogether 37 patients were receiving insulin and 3 cases were controlled with oral drugs at the time when the EEG tracings were obtained The average insulin requirement was 56 I U (range 28 to 128 I U) Diabetic retinopathy was present in three cases and diabetic nephropathy was diagnosed in one patient In addition different vascular manifestations such as hypertensive or arteriosclerotic heart disease (6 cases) coronary heart disease (2 cases) cerebral thrombosis (1 case) or marked peripheral vascular arteriosclerosis (1 cases) were present in some of the patients who were included in the study Patients with manifest clinical epilepsy were excluded from the material

The diabetic patients were divided into stable and labile cases according to the number of admissions to the hospital because of diabetic acidosis or hypoglycemic episodes and according to the blood glucose levels and the excretion of urinary glucose observed during visits to the out patient department Subjective complaints which were attributed to the occurrence of hypoglycemia had also to be taken into consideration in dividing the patients into these two categories Out of the total material consisting of 40 diabetics 22 patients were by means of the above mentioned criteria considered as stable cases whereas the diabetic state in 12 patients was regarded as moderately and in 6 patients as severely labile

The EEG recordings were performed with the aid of an eight-channel electroencephalograph with 16 electrodes Both monopolar and bipolar recordings were used The qualitative classification of the EEGs was performed according to the following commonly accepted criteria

P) *Paroxysmal activity* This group included EEG patterns showing bursts within the middle or slow (theta or delta) range spreading throughout the cerebral cortex

F) *Focal or local changes* In this group are included various types of alterations which are associated with focal lesions of the brain

D) *Diffuse changes* In this group belong the EEG patterns which exhibit diffuse changes representing alterations of normal electrocerebral activity without the characteristics of the two above mentioned groups

The quantitative assessment of the electrocerebral derangements was performed by rating the EEG alterations with respect to the three above mentioned features with the figures 0 1 2 and 3 The absence of EEG abnormalities was designated by zero (0) and mild moderate or severe changes of the EEG were designated by the figures 1 2 or 3 respectively These figures do thus not represent exact quantitative measurements of the EEG alterations but should rather be regarded as a semi quantitative measure of the various types of EEG alterations In addition all the EEG patterns of the diabetic patients were arranged both in order of increasing total amount of electrocerebral derangements and in order of increasing amount of paroxysmal activity, and the distribution of labile cases in these pre arranged series was determined by means of the Wilcoxon test All the classifications and arrangements of the EEG patterns was carried out by one of the investigators strictly without knowledge of the clinical criteria of the cases or the stability of the diabetic condition

Results

The EEG ratings obtained by the above mentioned method in three different age groups have in table I been added together, and 'ratings per case' have been obtained by dividing the total of the EEG ratings by the number of

TABLE I Correlation of EEG alterations in diabetic patients to the age of the patients

	Onset of diabetes								
	<40 yrs			41—60 yrs			>60 yrs		
	(24)			(10)			(6)		
	D	P	F	D	P	F	D	P	F
Total EEG ratings of the group	20	16	11	10	14	11	13	5	10
EEG ratings per case	0.83	0.67	0.46	1.0	1.4	1.1	2.2	0.83	1.67

D = diffuse changes P = paroxysmal activity F focal changes

TABLE II Correlation of EEG alterations in diabetic patients to the duration of diabetes

	Duration								
	0—5 yrs			5—10 yrs			>10 yrs		
	(9)			(15)			(14)		
	D	P	F	D	P	F	D	P	F
Total EEG ratings of the group	9	8	6	14	13	11	19	11	12
EEG ratings per case	1.0	0.89	0.67	0.93	0.87	0.73	1.36	0.79	0.86

D = diffuse changes P = paroxysmal activity F = focal changes

TABLE III Correlation of EEG alterations in diabetic patients to the stability of the diabetic condition

	Stable diabetics			Labile diabetics		
	(21)			(13)		
	D	P	F	D	P	F
Total EEG ratings of the group	14	13	10	22	14	13
EEG ratings per case	0.67	0.62	0.93	1.69	1.08	1.0

D diffuse changes P paroxysmal activity F focal changes

pitalized in the neuropsychiatric wards of the Hesperia Hospital, the Medical Department of the Kivela Hospital or the Third Medical Department of Helsinki University

The known incidence of diabetes in the relatives of the patients included in the study was 37 % (15 cases), and 2 relatives of the patients were known to have epilepsy The average age of the patients was 43 6 years (range 16 to 71 years) and the known duration of the disease 9 6 years (range 1 to 27 years) Altogether 37 patients were receiving insulin and 3 cases were controlled with oral drugs at the time when the EEG tracings were obtained The average insulin requirement was 56 I U (range 28 to 128 I U) Diabetic retinopathy was present in three cases and diabetic nephropathy was diagnosed in one patient In addition different vascular manifestations such as *hypertensive or arteriosclerotic heart disease* (6 cases) coronary heart disease (2 cases) cerebral thrombosis (1 case) or marked peripheral vascular arteriosclerosis (1 cases) were present in some of the patients who were included in the study Patients with manifest clinical epilepsy were excluded from the material

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Results

The EEG ratings obtained by the above mentioned method in three different age groups have in table I been added together, and "ratings per case have been obtained by dividing the total of the EEG ratings by the number of

No correlation was found to exist during the course of the investigation between the EEG alterations of the diabetic patients on the one hand and the occurrence of diabetic microangiopathy or clinical manifestations of cardiovascular disease on the other

Discussion

It has been well documented that both in man and in experimental animals the EEG is altered by changes in blood glucose levels This is probably a result of reduced oxygen utilization of the brain and the parallelism of the symptoms seen during hypoglycemia and anoxia has been pointed out by several authors (2) According to Himwich (10), five different phases which can be ascribed to differences in the oxygen and glucose demands by phylogenetically younger and older parts of the brain can be observed during progressive hypoglycemia

The brain injury which is caused by hypoglycemia can be reversible Thus Fabrykant (4) observed that improvement of initially abnormal EEGs recorded in diabetic patients who had experienced repeated severe insulin reactions was maintained during several years following discontinuation of anticonvulsive therapy which had been administered for a short period of time Several cases of irreversible brain injury due to hypoglycemia have on the other hand been reported in the literature (5 6 7 14) and the morphological changes of the brain have in most cases been similar to the pathological alterations observed in other conditions associated with anoxia (2, 7)

If brain injury due to various degrees of hypoglycemia were the main reason for the alterations of the EEG observed in diabetic patients these alterations could be expected to occur more frequently in the diabetics who experience frequent severe hypoglycemic episodes In most studies, however no strict parallelism has been found to exist between the EEG abnormalities on the one hand and the clinical severity or lability of the diabetic state on the other

Thus Fabrykant noted no significant correlation between the stability of diabetes and EEG alterations, although a marked distortion of the EEG was observed in patients with a history of severe insulin reactions (4) Izzo et al (11) on the other hand found no correlation of EEG abnormalities to the severity or instability of the diabetic state

During the course of the present investigation a significant increase of paroxysmal activity was observed in the diabetic patients who on the basis of clinical criteria were considered as labile cases Although the nature of paroxysmal EEG changes is still open to debate it is certain that these changes reflect lesions of the deep subcortical parts of the brain Such lesions can be produced by traumatic episodes or prolonged unconsciousness due to severe anoxia A history of severe hypoglycemia leading to prolonged unconsciousness could however not be elicited in most of the labile diabetics and it is well known on the other hand that abnormal

EEG tracings can be absent even in severe hypoglycemia states (9) and are infrequently encountered e g following profound insulin hypoglycemia induced during the treatment of psychiatric conditions Estimation of 'lability' must moreover in many instances depend on subjective complaints, which are thought to be due to hypoglycemia although e g variations in blood glucose levels which do not necessarily involve a decrease of blood glucose to hypoglycemic values can cause similar symptoms It has on the other hand been shown that electrocerebral changes during a fall in blood glucose levels are small until the glucose concentration in the internal jugular vein reaches a level of 30 mg % or less (2) Hypoglycemia like symptoms which are attributed to a fall of blood glucose concentrations within normal limits have by some authors been termed as pseudohypoglycemic reactions (3), and it cannot be excluded that the subjective complaints on the basis of which some of the diabetics of the present series have been categorized as labile cases have been due to such reactions

It would thus seem unlikely that the increased paroxysmal EEG activity observed in many of the diabetic patients included in the present study could — except in a few cases — be interpreted in terms of irreversible brain damage due to hypoglycemia Since similar paroxysmal changes are also found in subcortical forms of epilepsy, it could be postulated that the brain of a diabetic who exhibits this abnormality is unable to carry out *its usual stabilizing function* and that the paroxysmal derangements in the function of the subcortical parts of the brain could in some cases contribute to the lability of the diabetic state The known stabilizing effect of barbiturates and other antiepileptic drugs in the treatment of diabetics who are difficult to control seems to lend further support to this concept The existence of a 'primary' diabetic encephalopathy has in fact been postulated by some investigators (15), and the occurrence of EEG alterations in "healthy" relatives of diabetic patients (1) suggests the importance of genetic factors in the production of these abnormalities Since other abnormalities such as derangements of both carbohydrate and lipid metabolism can also be observed in "healthy" non diabetic relatives of diabetic patients (12), it would seem possible to attribute the brain damage which is manifested by the abnormal EEG tracings in these individuals (and possibly also in a part of the manifestly diabetic population) to metabolic alterations of the brain which do not necessarily involve profound hypoglycemia

Another factor which must be taken into consideration in the production of EEG alterations in diabetic patients is brain damage due to cerebrovascular lesions Thus Andersson and Kirstein (1) noted an increased incidence of EEG changes, and in particular focal lesions in diabetics with increasing age and duration of diabetes, these factors being known to influence the occurrence of vascular lesions They concluded that cerebrovascular lesions are the most important causal factor in the production *of pathological electrocerebral* activity in diabetic patients, although

abnormal EEGs in some diabetics evidently can be caused by post hypoglycemic brain damage

In view of the well known increase of EEG changes with increasing age which was also noted during the course of the present study, patients over the age of 60 were excluded from our series Although vascular lesions, of course, can be present even in younger diabetics and have been described also after a very short duration of the disease the age limit used in the present series would nevertheless seem to preclude at least to some degree brain damage of cerebrovascular origin as a cause of the observed EEG abnormalities Furthermore no correlation between the duration of diabetes and the amount of electrocerebral derangements could be observed during the course of the present investigation

The observations of Heik et al (9) which are based on 400 EEGs obtained in 156 diabetic children are also noteworthy in this context Diffuse or focal alterations were in this material noted in a total of 76 % of the diabetic children and it was pointed out by the investigators that EEG abnormalities were observed in diabetic children with a very short duration of the disease in whom no signs of diabetic retinopathy or nephropathy could be elicited This study would likewise seem to speak against the importance of vascular lesions in the production of the electrocerebral alterations in diabetes Pathological EEGs were moreover encountered in a few children with diabetes of recent onset who had not yet received insulin treatment and who had not experienced any hypoglycemic episodes These findings could be interpreted in terms of a 'primary' electrocerebral derangement in diabetes which is unrelated both to cerebrovascular lesions and post hypoglycemic injury of the brain

It can be concluded that EEG abnormalities in diabetic patients can be due to multiple reasons The observations made during the course of the present study support the view that probably only in severe cases does post hypoglycemic brain injury cause permanent alterations of the EEG pattern Clinical episodes of hypoglycemia even if repeated are in most cases not associated with any changes in the EEG, whereas EEG abnormalities are often encountered without the occurrence of clinical signs of hypoglycemia and can in these instances in the absence of brain injury of cerebrovascular origin probably be attributed to genetically determined or early acquired alterations at the cellular level in the brain

Summary

Electroencephalographic (EEG) tracings were obtained in 40 insulin dependent diabetic patients of whom 22 were according to clinical criteria stable cases and 18 were considered to be labile diabetics The EEG alterations observed in these patients were on a qualitative basis divided into diffuse, paroxysmal and focal changes and in addition a quantitative estimate of the electrocerebral derangements was obtained by rating the EEG patterns with respect to all three kinds of alterations into four groups representing absence of patho

logical changes or various degrees of electrocerebral alterations

All types of EEG alterations were found to increase with increasing age, although the electrocerebral derangements were not found to be correlated to the known duration of diabetes, the administered dose of insulin or the occurrence of clinically demonstrable vascular complications EEG alterations were on the other hand found to be generally more pronounced in the labile diabetics than in the stable cases, and a significant increase of paroxysmal electrocerebral activity was observed in the former

It is concluded that cerebral factors can contribute to the lability of the diabetic state Electrocerebral alterations in diabetic patients are on the other hand only rarely caused by hypoglycemic brain damage Abnormal EEG tracings in diabetic patients must in many cases probably be attributed to genetically determined or early acquired alterations of the brain although EEG changes particularly in older age groups also can be caused by brain injury of cerebrovascular origin

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From the Department of Pulmonary Diseases (Head E. von Rosen M D) and the Department of Clinical Chemistry (Head C G Holmberg M D) University of Lund Lund Sweden

Serum Proteins and Liver Function in Sarcoidosis[1]

By

R Ingestad and N Tryding

In patients with sarcoidosis decreased serum albumin and increased serum globulins are very commonly found (5, 15, 17, 19, 20, 24) This paper is mainly concerned with the electrophoretic serum protein pattern in different roentgenologic stages of sarcoidosis In an attempt to ascertain whether patients with sarcoidosis have a constitutional tendency to hypergammaglobulinaemia it was decided to examine the electrophoretic serum protein pattern, especially the gamma globulin concentration, in relatives of patients with sarcoidosis and with tuberculosis respectively In addition liver function in patients with sarcoidosis was studied with the bromsulphthalein test

Material

The material was selected at random from that used in an investigation by Ingestad and consisted of 115 patients with roentgenologic evidence of pulmonary sarcoidosis 50 males and 65 females Histologic examination of biopsy specimens from one or more organs revealed sarcoid tissue in 99 of the 115 patients The study also covered 93 siblings and 51 children of our patients. For comparison 53 siblings and 19 children of patients with tuberculosis were examined The sex and age distribution of the subjects is given in table IV Among the above-mentioned relatives 4 siblings of patients with sarcoidosis had allergic diseases (2 hay fever and 2 bronchial asthma None of the relatives had tuberculosis sarcoidosis or "collagen disease in their history General physical examination and radiography revealed no abnormalities of relevance

Methods

Paper electrophoresis was done according to Laurell et al (13) The normal ranges are given in table I The blood samples were collected in the fasting state The serum samples were stored at —20 C until analysed

The ESR was done according to Westergren s method in association with collections of the sample for electrophoresis

Liver function test The bromsulphthalein test was done with 5 mg per kg body weight

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TABLE I. Paper electrophoresis of serum from patients with sarcoidosis in different roentgenological

Group	N ♂	N ♀	Age m	Age s	Alb (g/100 ml) Mdn	Alb (g/100 ml) Variation	α (g/100 ml) Mdn	α (g/100 ml) Variation
Stage I	15	21	36.0	12.3	4.77	3.41–5.61	0.27	0.18 0.48
Stage II	18	22	43.4	14.1	4.45	2.64–5.93	0.32	0.20–0.68
Stage III	12	24	52.4	10.1	4.65	3.73–5.76	0.28	0.22–0.43
Healed	13	14	39.5	12.2	5.12	3.99–5.84	0.25	0.17–0.33
Normal ranges						4.8–5.8		0.[illegible]0–0.32

Statistical analysis
(Kruskal Wallis's one way analysis of variance)
(The Mann Whitney U test)

Albumin
H = 28.34 df = 3 P < 0.001

Stage
I—II z = 1.39 P > 0.05
I—III z = 0.10 P < 0.05
I—healed z = 3.44 P > 0.001
II—III z = 1.66 P > 0.05
II—healed z = 4.79 P < 0.001
III—healed z = 6.67 P < 0.001

α
H = 23.15 df = 3 P < 0.001

Stage
I—II z = 2.16 P < 0.05
I—III z = 0.34 P 0.05
I—healed z = 2.52 P < 0.05
II—III z = 1.84 P > 0.05
II—healed z = 4.51 P < 0.001
III—healed z = 2.76 P < 0.01

α_1
H = 18.17 df = 3 P < 0.001

Stage
I—II z = 1.37 P > 0.05

When retention exceeded 10 % after 30 min the test was said to be positive. The determination of alkaline phosphatase was done according to Bessey et al. (1) and serum bilirubin ad modum Nosslin (21).

The serum calcium was measured with an Eppendorf flame photometer.

Statistical analysis. The differences were examined by the X^2 test

$$X^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

Results

Serum protein electrophoresis

Altogether 139 samples from 115 patients with sarcoidosis were studied electrophoretically. The results are summarized in table I.

The serum albumin was abnormally low in all 3 roentgenological stages of the disease and was lowest in stage II. The difference observed in the albumin concentration between active sarcoidosis (stages I, II and III) and roentgenologically healed sarcoidosis was significant.

The alpha₁, alpha₂ and beta globulin concentrations were significantly higher in stages I, II and III than in the roentgenologically healed group. The highest individual concentrations were found in stage II.

stages

α_1 (g/100 ml)		β (g/100 ml)		γ (g/100 ml)		Total protein (g/100 ml)	
Mdn	Variation	Mdn	Variation	Mdn	Variation	Mdn	Variation
0 54	0 36–1 09	0 72	0 54–1 03	1 11	0 75–2 02	7 5	6 3–8 5
0 62	0 39–1 29	0 76	0 51–1 19	1 34	0 86–2 12	7 6	6 0–9 0
0 57	0 39–0 75	0 74	0 55–1 05	1 35	0 72–2 33	7 7	5 9–9 0
0 50	0 31–0 68	0 65	0 50–0 86	0 98	0 70–1 70	7 6	6 6–8 6
	0 32–0 54		0 43–0 85		0 61–1 17		6 8–8 2

I–III	z=0 36	P > 05
I–healed	z=2 08	P < 05
II–III	z=1 89	P > 05
II–healed	z=4 03	P < 001
III–healed	z=2 56	P < 05

β

H=18 76	df=3	P < 001
Stage		
I–II	z=1 74	P > 05
I–III	z=0 95	P > 05
I–healed	z=2 51	P < 05
II–III	z=0 57	P < 05
II–healed	z=3 73	P < 001
III–healed	z=3 06	P < 001

γ

H=27 64	df=3	P < 001
Stage		
I–II	z=2 59	P < 01
I–III	z=2 39	P < 02
I–healed	z=2 12	P < 05
II–III	z=0 33	P > 70
II–healed	z=4 56	P < 001
III–heeled	z=4 39	P < 001

P < 05 probably significant
P < 01 *significant*
P < 001 *highly significant*

The gamma globulin fraction was likewise increased in stages I II and III, *especially in stages II and III*

In the roentgenologically healed cases the median values found for the various fractions fell within the normal range of variation, but some of the individual globulin values exceeded the normal limits which suggested that the disease had not completely healed *despite the* absence of any roentgenographic changes in the lungs

The patients in stage I produced different clinical symptoms and two *principal groups may be distinguished*

1 Patients with acute onset of the disease with erythema nodosum

2 Patients with insidious onset of the disease without erythema nodosum

The serum gamma and beta globulin concentrations in patients belonging to these two groups were found to be largely the same The alpha globulins *were significantly higher and the albu min was lower in the group of erythema* nodosum (table II) Only patients in *whom the roentgenographic changes* regressed were compared

In several patients the roentgeno graphic appearance of the lungs varied

TABLE II. Serum proteins and ESR of sarcoid patients in stage I with and without erythema nodosum

	N		Age		Alb (g/100 ml)		α_1 (g/100 ml)	
Group	♂	♀	m	s	Mdn	Variation	Mdn	Variation
Stage I with E.N.	5	9	38.6	11.1	4.00	3.41–5.26	0.34	0.24–0.48
Stage I without E.N.	8	7	29.4	11.0	4.98	4.24–5.29	0.26	0.18–0.33

Statistical analysis

Albumin	$U = 36.0$	$P < 0.02$
α_1	$U = 34.5$	$P < 0.02$
α_2	$U = 48.0$	$P < 0.2$
β	$U = 105.0$	$P > 0.5$
γ	$U = 97.5$	$P > 0.5$

TABLE III. Serum proteins and ESR of sarcoid patients in stage II with progressive and regressive

	N		Age		Alb (g/100 ml)		α_1 (g/100 ml)	
Group	♂	♀	m	s	Mdn	Variation	Mdn	Variation
Stage II (→ III)	4	4	47.5	10.4	4.32	3.73–4.69	0.33	0.26–0.36
Stage II (→ healed)	7	10	38.2	14.1	4.46	3.38–5.95	0.32	0.23–0.43

Statistical analysis

Albumin	$U = 46.5$	$P > 0.5$
α_1	$U = 59.0$	$P > 0.5$
α_2	$U = 65.0$	$P > 0.5$

in the course of the investigation. In some cases in stage II the roentgenographic changes advanced to stage III while in others they regressed. In order to get a clinically equivalent comparison between progressive and regressive cases in stage II, only patients with insidious onset without erythema nodosum were included in the two groups. It is clear from table III that the gamma globulin concentration determined in stage II was generally somewhat lower in those cases in which the disease healed than in those in which it progressed to stage III but for none of the serum protein fractions is the difference significant. Thus the gamma globulin concentration in stage II appears to be of no prognostic significance in the individual case.

Erythrocyte sedimentation rate

In the patients with sarcoidosis the ESR showed a wide range of variation

α_1 (g/100 ml)		β (g/100 ml)		γ (g/100 ml)		ESR (mm/hour)	
Mdn	Variation	Mdn	Variation	Mdn	Variation	Mdn	Variation
0 69	0 36—1 09	0 73	0 57—0 87	1.20	0 79—2 02	71	40—124
0 49	0 37—0 76	0 72	0 54—1 03	1 11	0 75—1 98	10	4—33

ESR U=0 0 P < 001
P < 05 probably significant
P < 01 significant
P < 001 highly significant

course respectively

α_1 (g/100 ml)		β (g/100 ml)		γ (g/100 ml)		ESR (mm/hour)	
Mdn	Variation	Mdn	Variation	Mdn	Variation	Mdn	Variation
0 62	0 42 0 70	0 75	0 65—1 19	1 47	1 07—1 93	23	5 68
0 62	0 39 1 19	0 80	0 51—1 13	1.29	0 87—2 12	12	2—52

β U=68 0 P > 05
γ U−54 5 P > 05
(no significance)

(table IV) The median values were increased in all the 3 stages The highest individual values were noted in stage I In several of these active cases the ESR was normal The median ESR in the roentgenologically healed group was normal

In stage I the median ESR was significantly higher in patients with erythema nodosum than in those with an insidious onset of the disease (table II) The median ESR determined in stage II was largely the same in those patients in whom the disease healed as in those in whom it progressed to stage III (table III)

Findings in relatives of patients with sarcoidosis

The serum gamma globulin concentrations found in the patients with sarcoidosis in various roentgenologic stages as

TABLE IV Serum γ globulin values and ESR of patients with sarcoidosis in different roentgenological stages as well as of relatives of patients with sarcoidosis and with tuberculosis

Group	N ♂	N ♀	Age m	Age s	Serum γ globulins (g/100 ml) Mdn	Serum γ globulins (g/100 ml) Variation	ESR (mm/hour) Mdn	ESR (mm/hour) Variation
Patients with sarcoidosis								
Stage I	15	21	36 0	12 3	1 11	0 75–2 07	16 5	2–121
Stage II	18	22	43 4	14 1	1 34	0 86–2 12	20 1	2–81
Stage III	12	24	52 4	10 1	1 3ɟ	0 72–2 33	15 0	2–93
Healed	13	14	39 5	12 2	0 98	0 70–1 70	6 2	1–20
Siblings of sarc pat	45	48	40 8	14 0	1 01	0 57–1 60	6 4	1–36
Children of sarc pat	19	32	21 5	10 0	0 94	0 51–1 67		
Siblings of tuberc pat	23	30	39 6	11 3	0 97	0 68–1 64	6 0	2–28
Children of tuberc pat	10	9	25 3	15 1	0 93	0 66–1 36		

Statistical analysis
(The Mann Whitney U test)

	Comparison	z	P
γ	Stage I siblings of sarcoid patients	z 3 11	P < 002
	Stage II siblings of sarcoid patients	z=5 74	P < 001
	Stage III — siblings of sarcoid patients	z=6 69	P < 001
	Healed siblings of sarcoid patients	z 0 21	P ≈ 80
ESR	Stage I — II	z 0 56	P 50
	Stage I III	z=0 35	P 70
	Stage I healed	z=3 90	P < 001
	Stage II — III	z=1 43	P 10
	Stage II healed	z=5 29	P < 001
	Stage III healed	z 4 01	P < 001

P < 05 probably significant
P < 01 significant
P < 001 highly significant

well as in relatives of patients with sarcoidosis or tuberculosis are given in table IV

The median serum gamma globulin concentration in the roentgenologically healed patients did not differ significantly from that noted in the relatives of patients with sarcoidosis and tuberculosis respectively Each of these 3 groups included a few members with hypergammaglobulinaemia With but few exceptions the persons with hypergammaglobulinaemia did not belong to the same family Nor was any significant difference in the ESR found between the roentgenologically healed sarcoidosis group and the siblings of patients with sarcoidosis or tuberculosis

Liver function tests

Liver function was studied in 66 of the patients with sarcoidosis 25 males and 41 females None of them had any known disease of the liver or the heart In 20 of the patients studied the brom sulphthalein test was positive The positive tests were noted mostly in patients in stage II of the disease In table V the results of the bromsulphthalein tests are related to the serum alkaline phosphatase, to the serum protein values and to the results of histologic examination of biopsy specimens of the liver The serum alkaline phosphatase was found to be increased in 3 out of 38 patients studied In all 3 the disease was in stage II and the bromsulphthalein test was positive, but the serum bilirubin concentration was not increased in these or in other patients studied Abdominal palpation revealed no enlargement of the liver in any of the 66 patients

Liver biopsy ad modum Radner was done in 30 cases Among these, the bromsulphthalein test was positive in 11 and negative in 19 The biopsy specimen showed signs of sarcoid tissue in 6 cases The bromsulphthalein test was positive in 3 of these cases and negative in the remaining 3 All these 6 cases were in stage II or III There thus appeared to be no correlation between positive bromsulphthalein test and positive histologic findings in the liver Roentgenographic hepatomegaly was verified in 2 of the 30 cases examined In both cases the bromsulphthalein test was positive, but the liver biopsy specimen showed no sarcoid tissue A slight correlation between the brom sulphthalein test and the serum gamma globulin concentration was found The other serum globulins did not vary with the results of the bromsulphthalein test

Serum calcium content

Only in 2 of the patients studied (35 males and 52 females) was the calcium content found to be increased, and then only slightly (5 4 and 5 5 mEq/l) In one patient, who proved to have renal sarcoidosis, the serum calcium (5 2 mEq/l) bordered the upper normal limit Histologic examination of a biopsy specimen of kidney tissue showed the characteristics of sarcoid lesions and interstitial nephritis but no signs of nephrocalcinosis

Discussion

An increase of the total serum protein content in patients with sarcoidosis was first demonstrated by Salvesen (22) Harrel and Fischer (6) Harrel (7), and Seibert et al (23) found hyperproteinaemia and hyperglobulinaemia in patients with active sarcoidosis The abnormality disappeared when the disease healed Murray et al (19) studied 12 cases of histologically confirmed sarcoidosis in different stages In 4 of the patients with no signs of active disease the serum protein pattern was largely normal In patients with active sarcoidosis however, the concentration of the gamma globulin was increased while that of the albumin was decreased Sunderman and Sunderman (24) found by paper electrophoretic studies an increased concentration of total protein in the serum diminished concentration of albumin and step like

TABLE V Relation between liver biopsy liver function and serum proteins

Bromsulphthalein test	N		Age		Liver biopsy	Alk phosf	Serum protein con	
							Alb (g/100 ml)	
	♂	♀	m	s			Mdn	Variation
Positive (>10%)	7	13	50 8	16 7	3/11	3/12	4 55	?64–5 40
Negative (<10%)	18	28	41 1	10 9	3/19	0/26	4 78	3 12–5 ,6

Statistical analysis Bromsulphthalein test positive — bromsulphthalein test negative

increases in the concentrations of alpha₂, beta, and gamma globulin ('sarcoid steps)

Levitt (15) studied the paper electrophoretic protein pattern in 25 patients with histologically confirmed sarcoidosis The alpha₂ beta, and especially the gamma globulin fractions were found to be increased while the serum albumin was definitely decreased

Norberg (20) recently reported 82 histologically confirmed cases of sarcoidosis in which the serum protein pattern was studied in various stages of the disease according to the same principle as that used by us The total serum protein was increased in the patients with progressive lesions, but was normal or only slightly increased in those with stationary lesions In all the groups the mean relative albumin concentration was lowered The mean alpha₂-globulin level was elevated in all of the groups, especially in the progressive cases In several cases of each groups the beta globulin was also increased The mean relative gamma globulin concentration was increased only in the groups with progressive lesions

It is well known that the serum protein values are higher in ambulant than in non ambulant patients According to Lange (11), the difference is approximately 9 % In Norberg's study (20) this difference was 8 3 % in the controls and 14 5 % in the cases with sarcoidosis In our material, both the patients with sarcoidosis and the relatives, almost all the samples were taken from ambulant persons and therefore the results in the different groups may be considered comparable

Like Norberg (20), we found decreased serum albumin and increased alpha₂ and beta globulines in all stages of active sarcoidosis The gamma globulin concentration was especially elevated in stages II and III

The great ESR differences between patients in stage I with erythema nodosum and those with insidious onset is not correlated to any differences in the gamma globulin concentration The patients in the erythema nodosum group

centration							
α_1 (g/100 ml)		α_2 (g/100 ml)		β (g/100 ml)		γ (g/100 ml)	
Mdn	Variation	Mdn	Variation	Mdn	Variation	Mdn	Variation
0.30	0.21–0.68	0.57	0.36–1.29	0.80	0.51–1.05	1.47	0.79–2.21
0.28	0.20–0.44	0.54	0.37–1.04	0.74	0.57–0.93	1.19	0.83–2.12

α_1 α_2 β (no significance) γ $z = 2.21$ P = 027 (probably significant)

however, had significantly lower serum albumin and higher alpha globulin values

A comparison between progressive and regressive cases in stage II showed no significant difference to the serum protein fractions Thus like Greenberg et al (5) we found no prognostic significance in the serum protein values

Dieckmann (4) pointed out that granulomatosis of the sarcoid type can be seen in patients with agammaglobulinaemia According to him, the serum gamma globulin concentration is of no diagnostic or prognostic value in sarcoidosis

It is, however well established that hypergammaglobulinaemia is a common accompaniment of active sarcoidosis and of systemic lupus erythematosus (SLE) In an investigation of relatives of patients with SLE Larsson and Leonhardt (12) and Leonhardt (14) found hypergammaglobulinaemia to be fairly common in relatives of the patients It was concluded that the tendency to hypergammaglobulinaemia in the relatives of patients with SLE is probably a constitutional abnormality of the immunologic system As in SLE a familial occurrence of sarcoidosis has been reported (8, 9, 18) Our study of the serum globulin content in siblings and children of patients with sarcoidosis did not, however, produce any evidence of a constitutional tendency to hypergammaglobulinaemia in sarcoidosis

According to Branson et al (2), the liver is affected in 65 % of all patients with sarcoidosis Cheitlin and Sullivan Jr (3) surveyed published cases of liver cirrhosis due to sarcoidosis to which they added one of their own In our investigation the bromsulphthalein test showed impairment of liver function in 20 out of 66 patients examined in this respect Little correlation between the result of the bromsulphthalein test and the serum gammaglobulin concentration was found

Only in 2 out of 20 patients with sarcoidosis did roentgenography show hepatomegaly In neither of them did liver biopsy specimen show sarcoid

lesions Nor was any correlation found between the histologic findings in the liver biopsy specimens and the results of the bromsulphthalein tests

Summary

One hundred and fifteen patients with roentgenologic evidence of pulmonary sarcoidosis were subjected to tissue biopsy, mainly of the lymph nodes (scalene fat pad biopsy) Histological examination revealed sarcoid lesions in 99 of the patients Paper electro phoresis of the serum proteins in these patients showed a strongly significant increase of the globulin fractions during stage II and stage III of the disease In stage I the serum globulin values were only slightly elevated, and there was no difference in serum gamma globulin concentration between patients with and without erythema nodosum but the serum albumin was lower and the alpha-globulins higher in the group of erythema nodosum As a rule the roentgenographic and the electrophoretic abnormalities disappeared roughly simultaneously in those cases that healed Some patients were followed from roentgenologic stage II up to complete disappearance of the roentgenographic changes or to a stage of pulmonary fibrosis The values of the serum protein fractions and the ESR in stage II did not allow any prognostic conclusions

In order to check whether patients with sarcoidosis have any constitutional tendency to hypergammaglobulinaemia siblings and children of patients with sarcoidosis were studied with regard to the electrophoretic serum protein pattern For comparison, relatives of patients with pulmonary tuberculosis were also studied No significant difference was found in the concentration of the serum gamma globulins between the groups of relatives and the patients with roentgenologically healed sarcoidosis Thus, no evidence of familial occurrence of hypergammaglobulinaemia was produced

The liver function of 66 patients with pulmonary sarcoidosis was studied with the bromsulphthalein test The retention of the dye was increased in as many as 20 A correlation was found between impairment of liver function and the increase of the serum gamma globulin concentration

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From the Departments of Medicine (Head P Bechgaard M D) Surgery (Head P Holm Nielsen M D) and Diagnostic Radiology (Head Aa Wagner M D) Aarhus Amtssygehus Aarhus Denmark

Gastric Acid Secretion Related to Prognosis in Peptic Ulcer

A Long term Follow up Study

By

EINAR KRAG

The interest in studies of gastric acid secretion was greatly stimulated when in 1953 Kay (12) published his augmented histamine test This quantitative method of analysis is stated to be superior to those previously used with regard both to stimulation of the parietal cells and to accuracy and reproducibility

By means of the augmented histamine test it is possible to study accurately the diagnostic significance of the maximum acid secretion in various gastric disorders especially peptic ulcer, gastritis and cancer of the stomach (1, 2 7, 11 16)

The prognostic significance of the acid production has not been systematically studied but in 1935 Emery and Monroe (9) showed that gastric acidity as such did not influence the prognosis in a 4 year follow up study of 994 patients with peptic ulcer In 1963, Bockus (4) wrote Although sufficient statistics are not available it is likely that duodenal ulcer patients with hyper secretion and grade IV acidity (free acid above 100 mEq/l) have a somewhat poorer prognosis both from the standpoint of recurrence and the likelihood of development of complications

The purpose of the study reported here was to throw light on the prognostic significance of gastric acid secretion in peptic ulcer by means of Ewald s test meal

Material and methods

During the period from 1936 to 1945 inclusive 251 patients with duodenal ulcer and 58 with gastric ulcer were treated in the Department of Medicine Aarhus Amtssygehus The treatment consisted in a restricted diet extending over 4—6 weeks bed rest and antacids (alkaline powder often combined with atropine)

First admission in 1936—1945

Barium meal examination including a study of the mucosal relief was performed in all cases Coarse irregular mucosal folds of the body and fundus of the stomach were observed in 61 of the 251 patients with duodenal

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TABLE III Follow up 1963 Relation between gastric acid secretion determined by the method of Ewald expressed by Kemp's index in ml of 1/10 N HCl in 1 hour on the first admission and the clinical course during the observation period

	Duodenal ulcer				Gastric ulcer			
	Gastric acid secretion				Gastric acid secretion			
	>100		<100		>100		<100	
Clinical course	(No)	(%)	(No)	(%)	(No)	(%)	(No)	(%)
Favourable	26	21	32	30	4	22	15	44
Less favorable	15	12	21	19	2	11	9	27
Serious	81	67	55	51	12	67	10	29
Followed up and Ewald tested total	122	100	108	100	18	100	34	100
Followed up but not Ewald tested		19				6		
Not followed up		2				0		
Total		251				58		

It appears from table I that the acid secretion was significantly higher in patients with duodenal ulcer than in those with gastric ulcer ($p < 0.02$)

Acid secretion and coarse mucosal folds Table II shows that in patients with duodenal ulcer an increased acid secretion is more frequently found when there are radiographically demonstrable coarse irregular mucosal folds of the body and fundus of the stomach than when there is a normal mucosal relief The difference is significant ($p < 0.05$) Similar conditions could not be demonstrated in patients with gastric ulcer

Follow-up examination

The patients were followed up in 1963 after observation periods varying from 17 to 27 years Information was obtained concerning 99 % of the patients

All the patients were followed to death or operation whilst those who were alive and had not undergone gastric operation were requested to return to the hospital for an interview

As regards the patients who had died before the follow up examination information as to the course of the ulcer disease and the cause of death was obtained through the patients own doctors and by means of death certificates

The hospital records for all patients who had been admitted to hospital for medical or surgical treatment of the ulcer disease during the observation period were reviewed

Clinical course On the basis of the clinical course of the ulcer disease the patients were at the follow up examination divided into three prognostic groups

Group A Favourable course

Complete freedom from symptoms or only slight dyspepsia not requiring any treatment throughout the observation period

Group B Less favourable course

One re admission and/or one manifest bleeding and/or recurrence of ulcer associated with incapacity for work

Group C Serious course

Two or more re admission and/or manifest bleedings or gastric operation or death from the ulcer disease

TABLE IV Follow up 1963 Relation between gastric acid secretion determined by the method of Ewald expressed by Kemp s index in ml of 1/10 N HCl in 1 hour on the first admissi n and the frequency of gastric operation during the observation period

	Duodenal ulcer 1936—1945				Gastric ulcer 1936—1945			
	Gastric acid secretion				Gastric acid secretion			
	>100		<100		>100		<100	
	(No)	(%)	(No)	(%)	(No)	(%)	(No)	(%)
Operation performed	62	50	35	32	5	28	7	21
Operation not performed	61	50	74	68	13	72	27	79
Followed up and Ewald tested total	123	100	109	100	18	100	34	100

TABLE V Follow up 1963 Relation between gastric acid secretion determined by the method of Ewald expressed by Kemp s index in ml of 1/10 N HCl in 1 hour on the first admission and the frequency of perforation during the observation period

	Duodenal ulcer 1936—1945			
	Gastric acid secretion			
	>100		<100	
	(No)	(%)	(No)	(%)
Perforation occurred	9	7	4	4
Perforation dit not occur	114	93	105	96
Followed up and Ewald tested total	123	100	109	100

Results

Acid secretion and clinical course Table III shows that patients with a high acid secretion (i e over 100 ml/10 N HCl in 1 hour as determined by the method of Ewald) on the first admission had a poorer prognosis during the observation period than those with a lower acid secretion The difference is significant For the entire series, $p < 0\ 01$, if patients with duodenal ulcer and those with gastric ulcer are considered separately, $p < 0\ 05$ in each of the two groups

Acid secretion and frequency of gastric operation As appears from table IV, patients with high acid secretion on the first admission had a higher frequency of gastric operation during the observa

TABLE VI Follow up 1963 Relation between gastric acid secretion determined by the method of Ewald expressed by Kemp s index in ml of 1/10 N HCl in 1 hour on the first admission and the frequency of *manifest bleeding during the observation period*

	Duodenal ulcer 1936—1945			
	Gastric acid secretion			
	>100		<100	
	(No)	(%)	(No)	(%)
Bleeding occurred	22	18	33	30
Bleeding dit not occur	101	82	76	70
Followed up and Ewald tested total	123	100	109	100

tion period than those with low acid secretion The difference is significant only for duodenal ulcer ($p < 0.01$)

Acid secretion and perforation Table V suggests that duodenal ulcer patients with high acid secretion on the first admission will have a higher frequency of perforation during the observation period than those with low acid secretion However the difference is not significant

Acid secretion and haematemesis/melaena Table VI shows that duodenal ulcer patients with high acid secretion on the first admission had a *lower* frequency of haematemesis/melaena during the observation period than those with low acid secretion The difference is significant ($p < 0.05$) No such relationship could be demonstrated in gastric ulcer

Discussion and conclusions

In this study the gastric acid secretion was determined by the method of Ewald As already mentioned, the results obtained by this method are reproducible only to a certain extent However, in 1960, Marks and Shay (17) published a comparative study of the gastric acid secretion determined by the augmented histamine test and by Ewald s test meal respectively, in 114 patients with various gastric disorders *The two authors* concluded that, in the group as a whole, there was a significant correlation between the results obtained by the two *methods On the other hand, if the groups* with duodenal or gastric ulcer were considered separately or if the patients were taken individually there was no *definite correlation between the results* of the two tests

Thus it is justifiable to draw cautious conclusions from the results based on *Ewald s test meal performed on large* groups of patients In this connexion it should be noted that in the present study the patients with duodenal ulcer *had a significantly higher acid secretion* than those with gastric ulcer although there was a certain amount of overlap between the two groups In principle, *this result accords with those from similar*

determinations of gastric acid secretion based on the augmented histamine test (1, 2, 7, 12).

In the present study, a correlation was revealed between high acid secretion and radiographically demonstrated coarse, irregular mucosal folds of the body and fundus of the stomach in duodenal ulcer patients. This is in agreement with a study published by Burns and Laws (5) in 1966. In 43 patients with duodenal ulcer they found a definite correlation between radiographically demonstrated coarse mucosal folds of the stomach and/or duodenum and high acid secretion as determined by the augmented histamine test. In this connexion it may be mentioned that Krag (15) observed a significant relationship between coarse mucosal folds and a poor clinical prognosis in 483 patients with peptic ulcer.

A correlation between the mucosal relief and acid secretion might, perhaps, be expected if the relief seen on the radiographs is interpreted as an expression of the actual size of the mucosa. In 1952 Cox (8) demonstrated a positive relationship between the size of the mucosa of the fundus and the number of parietal cells in stomachs studied at autopsy. In 1960, Card and Marks (6) found a correlation between the parietal cell mass and the maximum acid secretion and in 1962 Myren and Semb (18) showed that a correlation existed between the parietal cell mass and the basal acid secretion.

The results of the present study lend support to the assumption that high gastric acid secretion is indicative of a poor prognosis in patients with peptic ulcer. This assumption seems, in particular, to apply to duodenal ulcer patients, who in the presence of high acid secretion have not only a poor clinical prognosis, but also an increased frequency of gastric operation and possibly of perforation.

The tendency to haematemesis/melaena seems to be reduced in the presence of high acid secretion, which suggests that the acid production does not play the same pathogenic role for this complication as for the clinical course of the ulcer disease in general.

The conclusion of the present study must be that gastric acid secretion seems to be of great prognostic importance in the ulcer disease. The aim must be to improve the certainty with which the prognosis can be made in the individual patient. Future analyses based on the results of the augmented histamine test will show whether it is possible to attain this aim.

Summary

A follow up study concerning the relation between gastric acid secretion and the clinical course of peptic ulcer was performed on 309 ulcer patients from the period 1936—1945 who had received conservative treatment, viz. 251 patients with duodenal ulcer and 58 with gastric ulcer. The observation periods ranged from 17 to 27 years, and information was obtained concerning 99 % of the patients.

1. Patients with duodenal ulcer had a significantly higher acid secretion than those with gastric ulcer.

2 In patients with duodenal ulcer there was a significant correlation between the occurrence of radiographically demonstrated coarse, irregular mucosal folds of the fundus and body of the stomach and a high gastric acid secretion

3 High gastric acid secretion on the first admission to hospital is suggestive of a significantly poorer clinical course during the observation period than is low acid secretion This applies to both duodenal and gastric ulcer patients

4 High gastric acid secretion on the first admission in patients with duodenal ulcer suggests a higher frequency of gastric operation than does low acid secretion

5 High gastric acid secretion on the first admission in patients with duodenal ulcer involves a greater tendency to perforation during the observation period

6 High gastric acid secretion on the first admission is associated with a significantly *lower* frequency of haematemesis/melaena during the observation period than is low acid secretion

As was customary during the period in question, gastric acid secretion was determined by Ewald's test meal The reproducibility and accuracy of this method and its relation to the augmented histamine test are discussed

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From the Med cal Clin c B (Renal Cl n c) (Head N ls Alwall M D) and the Urologic Unit (Head Gosta Jonsson M D) of the Department of Surgery (Head Phil p Sandblom M D) Un vers ty of Lund Lund Sweden

Clot Retention in the Kidneys as a Probable Cause of Anuria During Treatment of Haematuria with Epsilon aminocaproic Acid

By

Gunnar Lingardh and Lennart Andersson

Epsilon am nocaproic acid (EACA) has in recent years been increas ngly used in the treatment of haematuria The ind cations have been bleeding in the lower urinary tract (3 10) as well as some forms of renal haematuria (2) McNicol (9) and Stark et al (15) have reported clot retention in the kidneys during treatment of haematuria and haemophil acs The following is an account of a pat ent with massive renal haematuria due to a coagulation defect in whom the bleeding was arrested by correct on of the coagulation defect and treatment with EACA but who developed anuria probably as a result of clot retent on in the kidneys

Case report

The pat ent a 59 year old woman had undergone surgery 8 years prev ously for suspected pancreat c cancer The operat on ncluded part al pancreatectomy cholecyst ectomy subtotal gastrectomy choledocho-jejunostomy gastrojejunostomy and pancreat cojejunostomy (mod fied Whipple s operat on) The pancreat c les on was not a car c noma but a callous gastric ulcer penetrat ing into the pancreas After th s operat on the pat ent developed steatorrhoea, passing 3—6 loose stools per day She was admitted to hosp tal on several occcas ons because of we ght loss wh ch necess tated parenteral nutrit on Invest gat on revealed a malabsorpt on syndrome includ ng defective absorpt on of v tam n B_{12} (the Schill ng test showing 4 % excret on over a 24-hour period) hypoprote naem a (total prote n occas onally down to 4 % usually below 5 5 % with a decrease *throughout all the fractions*) and greatly increased fat content in the faeces (24 hour excret on 45—85 g total fat 60—80 % the rat o fatty ac ds neutral fat being normal) The serum levels of iron calcium and phosphorus were w h n the normal range and l er tests were usually normal except for a temporar ly sl ghtly increased bromsulphale n retent on on a few occas ons She was g en a low fat h gh prote n d et pancreat c enzymes and vitam n B nclud ng B

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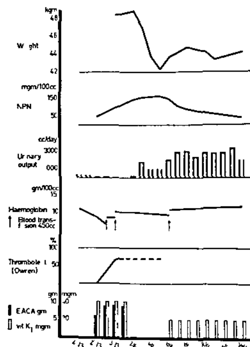


Fig 1 Survey of the case described

On March 27 1965 the patient was admitted to hospital after being confined to bed for 10 days because of an upper respiratory tract infection with nasal catarrh a dry cough and temperatures of 38—40° C Since the day before admission she had passed heavily blood stained urine without clots and had slight pain in the right flank radiating into the right groin Plain X rays showed findings consistent with bronchopneumonia in the right lung On admission she had no subcutaneous haemorrhages but after sampling blood continued to ooze from the puncture sites around which several subcutaneous haematomas developed within a few days The urine was massively intermixed with blood Plain X rays showed no concretions in the urinary tract the kidneys were of normal size On March 27 haemoglobin was 11 1 g/100 ml erythrocyte count 3,500 000, white cell count 15 000 and thrombocyte count 474 000 per mm^3 Haemoglobin fell during the days of bleeding (fig 1) Differential count was normal Thrombotest value on March 29 was below 5 % The bleeding time exceeded 30 min (Duke) and the clotting time in glass tube exceeded 45 min On March 29 treatment with vitamin K$_1$ was started, the daily dose being 40 mg intramuscularly and with EACA 6—9 g intravenously or orally, in doses as indicated in fig 1 The bleeding tendency disappeared quickly but the urine volumes, which had been satisfactory up to then diminished and anuria developed A bladder catheter was introduced but the bladder proved to be empty The catheter was left in position for a few days Because of a rising non protein nitrogen (N P N) *and serum potassium, and since she had an* attack of incipient lung oedema after 200 ml of mannitol 15 %, the patient was transferred to this hospital on April 2 EACA treatment was stopped Retrograde pyelography was planned but was not performed because urine secretion started spontaneously 36 hours after the last EACA dose The urine was at first dark brownish red and contained small fragments of blood clots It became clear within a few days The rest of the course will be seen from the figure N P N became normal creatinine clearance was 121 ml per minute on April 20 and the patient was discharged from the hospital *in good condi*tion on April 22

Discussion

The patient had steatorrhoea with malnutrition, manifesting itself by hypoproteinaemia which was accentuated in association with an acute infection during which a severe coagulation defect developed In this case the cause of the coagulation defect can be *attributed* to impaired absorption of vitamin K Defective absorption of the fat soluble vitamin K from the gastrointestinal tract can occur in the absence of bile (5) in some types of diarrhoea and gastrointestinal short circuits (6), and

in association with generally impaired fat absorption as in steatorrhoea due to pancreatic insufficiency, and in idiopathic steatorrhoea (11, 13, 14). Defective absorption of vitamin K gives rise to bleeding tendency because of reduced synthesis of factors II, VII, IX, and X (7, 8) and is mainly of the same type as that in dicoumarol intoxication. The commonest bleeding manifestation in idiopathic steatorrhoea seems to be ecchymosis, but haematuria, haemarthrosis, and gastro-intestinal bleeding are not rare (4).

In this case several factors may have played a part in causing the vitamin K deficiency. Defective absorption of fat prevents normal absorption of the fat-soluble vitamin K, which is synthesized by normal intestinal bacteria. A change in the nature of the intestinal contents and motility may have interfered with the production of vitamin K. Hypermotility of the intestine, with a shorter time for absorption, as well as reduced food intake during the acute infection may also have been causative factors.

During treatment with EACA and vitamin K in this case the clotting time became normal and the oozing of blood from the puncture sites ceased. The thrombo-test value rose from less than 5 % to 38 % in 24 hours after injection of 40 mg of vitamin K_1, and after another 24 hours it was over 65 %. EACA was continued for 4 days, the daily dose being 6—9 g orally or intravenously. The urine volumes diminished greatly, anuria developed and persisted right up to about 36 hours after the patient had received the last EACA dose.

There are many possible explanations to be considered as the cause of anuria in this case. It was not a matter of a simple obstruction of the flow from the bladder, as was ascertained by the introduction of an indwelling catheter. Acute glomerulonephritis can lead to rapidly transient anuria, but the prompt return of profuse urine secretion, as well as the absence of proteinuria and cylinder casts in the urine both during the patient's illness and at subsequent check-ups, argue against such an origin. The possibility of tubular nephritis must be carefully considered. Although clinical shock does not seem to have occurred, a tubular nephritis could have been induced by a combination of infectious intoxication and blood loss. The fact that the N.P.N. level, which was 154 mg/100 ml when secretion of urine started, did not become normal until the 10th—11th day of high daily urinary output (around 3 l) argues for the presumption that a lesion of the renal parenchyma was also present at that time. However, in cases of tubular nephritis the secretion of urine often starts more gradually than it did in this case, even though a fairly quick post-anuric return can occur. The absence of proteinuria need not exclude tubular nephritis, as proteinuria associated with this disease will usually disappear when N.P.N. has fallen to around 100 mg/100 ml (1).

In this case there was a marked relationship between the onset of anuria and the time of correction of the coagulation defect with vitamin K_1 during EACA treatment. In view of this we consider it more probable that clot

retention in the kidneys with resulting obstruction of urinary flow was the main cause The prompt return of profuse secretion of urine and the passage of old clot fragments over the next few days reinforce this argument As was mentioned above despite the large urine volumes the patient had post anuric impairment of renal function This would probably be adequately explained by the clot retention, with stasis gradually regressing during the clot lysis phase, together with the par enchymatous lesion thus caused Renal biopsy, after correction of the coagula tion defect, would have been of great interest, but unfortunately was not done

The fibrinolytic activity of urine, caused by its content of the plasminogen activator, urokinase, sustains bleeding of all kinds in the urinary tract (2, 10, 12) The greater part of orally or intra venously administered EACA is excreted unchanged in the urine, where it inhibits the activity of urokinase This accounts for the beneficial effect of EACA on urinary tract bleeding Shortly after the termination of treatment the urine regains its normal fibrinolytic activity and will again be able to lyse blood clots even those that are intermixed with EACA (2) This was probably the reason why secretion of urine re started in this patient As a rule, bleeding into the bladder in prostatic cancer and prostatic hyperplasia, as well as after operations on the prostate, ceases prompt ly on treatment with EACA In such cases clots are only rarely retained in the bladder If clots should appear in the bladder, they can easily be removed by means of a catheter or cystoscope

We have also used EACA success fully in several cases of mild or moder ately profuse bleeding from the kidneys, so called essential haematuria, and of post traumatic haematuria In cases of profuse bleeding from the kidneys, in which there is reason to fear that the renal pelvis will be filled with clots, the treatment is not without danger, as the inhibition of the fibrinolytic activity of the urine prevents the fragmentation of blood clots and thereby hampers their spontaneous passage In treating renal haematuria with fibrino lytic inhibitors it is important to check that the patient has a high urinary out put

Though EACA is a valuable remedy in the treatment of some forms of renal haematuria, its main field of usefulness is in bleeding from the lower urinary tract

Summary

A case is described of acute haemorrhagic diathesis attributable to malabsorption and associated with a very low thrombo test value The bleeding occurred main ly from the urinary tract and ceased when the patient was given vitamin K_1 and epsilon aminocaproic acid (EACA) Anuria developed Secretion of urine started again when EACA was discon tinued The anuria was probably caused by clot retention in the kidneys The explanation of this is presumed to be that the plasminogen activator of urine urokinase, was inhibited by EACA so that the urine lost its normal ability to dissolve clots The fibrinolytic activity of the urine was promptly restored when the EACA treatment was stopped

Acknowledgements

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From the Coagulation Laboratory (Head Inga Marie Nilsson M D) and the Department of Medicine (Head Jan Waldenstrom M D) University of Lund Allmänna Sjukhuset Malmo Sweden

Occurrence of Arteriosclerosis in von Willebrand's Disease

By

Jorgen Silwer, Stig Cronberg and Inga Marie Nilsson

According to Duguid (7 8, 9) atheromatous plaques can arise as the result of organization of thrombotic deposits on the vascular wall This theory has since received support from some experimental (10) and clinical (5) findings, but several objections have also been raised (24)

Investigations of patients with congenital haemorrhagic diathesis for arteriosclerotic changes should help to shed light on the pathogenesis of arteriosclerosis The condition has, for example been described in patients with haemophilia A and B (1, 2, 4, 13, 25) the bleeding time and platelet adhesiveness are normal in these coagulopathies but it cannot be excluded that platelets adhere to a damaged vascular wall and that they are afterwards transformed to atherosclerotic plaques

In another haemorrhagic condition von Willebrand s disease the bleeding time is prolonged owing to deficiency of an antibleeding factor ("von Willebrand factor) and factor VIII is decreased (14 15, 17) It has been postulated that the antibleeding factor is of importance in the initial phase of haemostasis (11) It was therefore thought to be of interest to find out whether arteriosclerosis can occur in this disorder

This paper reports an investigation of our Swedish series of patients with von Willebrand s disease for signs of arteriosclerosis

Material and methods

Clinical material

The diagnosis of von Willebrand s disease was made according to the definition of the disease given by Nilsson and Blomback (16 i e prolonged Ivy bleeding time AHF deficiency and an autosomal hereditary pattern A register is kept of all known cases in Sweden and at present we know of more than 200 cases belonging to 84 families Of the registered patients 6 were known to have died Of these 4 were more than 40 years old at the time of death and 3 of them were examined post mortem We have had the opportunity of examining the necropsy protocol in these 3 cases We also examined

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31 living patients above 40 years with a diagnosis of von Willebrand s disease The investigation was limited to patients above 40 years since clinical signs of atherosclerosis are rare below this age The family history, symptoms and coagulation status of most of the patients have been described previously (6 15, 17 18 22) These patients are referred to by the same initials, year of birth and family number as in the earlier papers The patients not described in earlier papers are referred to by the family number given in a survey of all the Swedish cases of von Wille brand s disease (to be published)

Methods used in coagulation studies

Blood sampling determination of the bleed ing time measurement of factor VIII and platelet adhesiveness were performed in the same way as in previous investigations (6, 22)

Investigations for arteriosclerosis

The patients were asked whether thev had had myocardial infarction thrombosis of the leg or any other cardiova cular disease They were also asked whether they had had chest pain and if so whether it radiated down the arms or up the neck and whether it was associated with physical exertion or exposure to cold The patients were also questioned concerning symptoms of cardiac insufficiency

In the search for arteriosclerosis of the legs the patients were asked whether thev had had leg pain which occurred on exertion particularly when walking and which ceased when they stood still or rested i e pains of intermittent claudication All other types of leg pain such as that often reported to occur in the evening after the patient had been standing or walking much during the day time were ignored

The patients were also asked whether they ever had headache dizziness balance disorders or memory difficulties These symptoms are however so unspecific and common that they permitted no conclusions

The clinical investigation consisted of physical examination, laboratory studies and roentgen examination The patients were examined for signs of cardiac incompensation and disturbances of the peripheral circula tion The heart was auscultated and the heart sounds cardiac rhythm and murmurs if any were noted The systolic blood pressure and the diastolic blood pressure were measured after 10 minutes rest in the usual way with application of a cuff The values for the diastolic blood pressure were noted when the sounds began to become less loud, and when they had disappeared The dorsal artery of the foot was palpated The ocular fundi were examined, often also by an opthalmologist

The urine was examined for glucose and albumin

Electrocardiography was done in all cases, mainly the resting ECG with standard leads unipolar limb leads and chest leads The chest leads used were mostly V leads but in a few patients CR leads were used instead according to the practice of the hospital In those cases where the patients reported symptoms suggesting coronary in sufficiency the ECG was recorded also after exercise on a cycle ergometer Con ventional electrocardiographic criteria of coronary insufficiency were used e g those described by Simonsson (23)

The heart abdominal aorta and limbs were examined roentgenographically for calcifications of the blood vessels and signs of heart disease The roentgenograms were examined by a roentgenologist (Dr Nils Frostberg)

Serum cholesterol was determined

Results

Post mortem examination had been perform ed on 3 patients above 40 years of age with von Willebrand s disease These cases are reported below

Case 1 G A fam 42 II 2 female born 1891 dead 1960 was the daugther of 42 I 1 O A who was troubled by severe nose bleedings. Clinical examination and laboratory studies had given a firm diagnosis of von Wille

brand s d sease n 5 of her 8 s bl ngs n 4 of her 9 children and in 6 of her 18 grand children She herself had had severe nose bleed ng and prolonged uter ne bleeding after an abort on n 1972 Analys s of her records then showed that the haemoglob n had fallen to less than 1 6 g/100 ml and the RBC to 960 000/mm³

S nce 1951 the pat ent had had period c chest pa n and dur ng the last year of her l fe in 1960 she had precordial pain on slight exert on On Dec 26 1960 she was adm tted to hosp tal w th the cl n cal d agnos s of myocard al nfarct on She d ed the following day

Necropsy revealed fresh nfarct on n the lef ventr cular wall and n the septum The myocard um as also otherwise of abnormal appearance and ga e the impress on of widespread prev ous ischaem c changes The coronary vessels showed severe atherosclerot ic changes and already a few cent metres from the r or g ns all 3 branches were almost totally occluded part cularly the descend ng branch The bottom of the aort c val e was moderatly calc fied and the aort c arch showed atheromatous nodules

Case 2 E S fam 41 male bo n 1892 dead 1964 belong ng to a fam ly without known p e ous cases of haemorrhagic d sease However 2 of the pat ent s 4 brothers had had ep sodes of severe nose bleed ng The pat ent h mself had always had an ncreased bleed ng tendency man festing itself espe c ally by severe nose bleed ngs and ready bru sab l ty In 1956 he was admitted to hosp tal because of melaena of unknown or g n and nose bleed ng w th consequent anaem a Coagulat on stud es showed pro- longat on of the bleed ng t me to between 6 and 11 m n according to Duke and 15 m n accord ng to Ivy The AHF content was decreased and var ed between 48 and 50 % The platelet count and other coagulat on factors were normal The pat ent s bleed ng symp oms ere d agnosed as s gns of von W llebrand s disease

S nce 1954 the pat ent had had known a ter al hypertens on wh ch var ed n recent years bet een 190 100 and 280 130 He was admitted to hosp tal in 1960 and 1962 because of r ghts ded hem pleg a He as readmitted n August 1964 because of repeated severe attacks of precord al pa n Electrotrocard ography and laboratory data corroborated the cl n cal d agnos s of myo- card al infarct on While in hosp tal he first improved b it later h s chest pa n and dyspnoea deter orated He d ed from card ac standst ll on Oct 7 1964

Necropsy showed pronounced general ath eroscleros s w th severe aort c atheromatos s and advanced atheroscleros s of the basal cerebral arteries Encephalomalac a was seen on the left s de of the bra n stem and hazel nut s zed cysts were found n the right occ p tal lobe Stenos ng coronary scleros s w th a small fresh thrombus was seen n the left descend ng branch Older fibrot cally healed infarct ons undergo ng resorpt on were found in the anter or wall of the left ventricle in the lateral wall and in the septum There were s gns of card ac nsuffi ciency w th pulmonary oedema b lateral hydrothorax and congest on

Case 3 N A fam 42 II 8 male born 1908 dead 1962 had in 1950 mass e bleed ng af er tooth extract on In the autumn of 1961 he was adm tted to hosp tal because of haematemes s and melaena He was g en 2 1/2 1 of blood Roentgen exam nat on of the oesophagus stomach and duodenum showed noth ng remarkable Exam nat on in May 1962 revealed a prolonged bleeding t me 15—24 min accord ng to Duke and more than 30 min accord ng to Ivy Factor VIII was decreased to 30 %

The pat ent d ed in Sept 1962 n an acci dent in wh ch he was crushed under a hea y l ft of a cement mixer and the aorta as lacerated Necropsy revealed solated yellow uncalc fied depos ts n the valls of the coronary arteries The aort c wall conta ned small yellow depos ts without calc ficat ons The cerebral arteries ere of normal ap- pearance

Exan nation of 31 pat ents with von W lleb and s disease abo e 40 years and st ll li ing re ealed no abnormalit es in 17

TABLE I Results of investigation for arteriosclerosis in 31 patients with von Willebrand's disease

Family no	Co-ordinate no	Case	Sex	Year of birth	Angina pectoris	ECG signs of coronary disease	B P (mm Hg)
2	III 15	WM	♂	1899	0	0	120/85–80
	III 21	HFN	♀	1907	0	0	120/90–75
	III 24	KM	♂	1911	0	0	125/95–80
	III 25	TM	♂	1911	0	0	120/100–100
	III 26	IL	♀	1914	0	0	115/80–75
4	III 6	AB	♀	1912	0	0	115/90–80
5	IV 5	PP	♂	1919	0	0	115/85–80
11	III 1	GB	♂	1913	0	0	110/85–80
14	II 4	BJ	♂	1911	0	0	115/75–70
18	IV 11	VÅ	♀	1924	0	(+)	150/100–90
20	III 1	GP	♂	1909	0	0	130/90–80
25	III 2	GL	♀	1918	+	+	175/105–105
34	III 7	SOP	♀	1914	0	0	120/90–85
35	IV 5	PT	♂	1905	0	0	150/100–90
42	II 1	EN	♀	1888	0	(+)	170/90–80
	II 4	HN	♀	1899	0	0	150/110–100
	II 5	ASB	♀	1903	0	0	120/90–80
	II 7	IA	♂	1906	0	0	120/90–80
	II 9	SP	♀	1912	+	0	140/110–100
	III 2	IN	♂	1913	0	0	150/95–90
	III 3	MR	♀	1917	0	(+)	150/110–100
	III 4	AF	♀	1923	0	0	150/110–100
	III 6	AGK	♀	1921	0	0	130/100–90
	III 19	IH	♀	1918	0	0	150/95–90
49	IV 1	AE	♀	1913	+	+	145/110–100
	IV 3	AO	♀	1917	0	0	155/110–100
54	III 7	MD	♀	1920	0	0	190/110–100
65	IV 1	BS	♀	1922	0	0	110/90–85
66		MS	♀	1915	0	0	130/100–90
81		NF[1]	♀	1902	++	+	250/150–140
88		IH	♀	1916	0	0	145/90–80

[1] Operated upon for arteriosclerotic gangrene of right foot
[2] Moderately sclerotic vessels in ocular fundi

while the remaining 14 were found to have symptoms or signs suspicious for arteriosclerosis The results are summarized in table I, and additional data are given below of the cases with possible signs of arteriosclerosis

WM fam 2 III 15, male, born 1899 In 1960 he complained of pain in the right foot The large toe was cold and numb Gangrene was diagnosed In 1963 the patient was subjected to transmetatarsal amputation of the right foot The wound would not heal He afterwards had pain and swelling of the

Cholesterol/s (mg/100 ml)	Calcifications				Dorsal art of foot	
	Thor aorta	Abd aorta	Other intra abd vessels	Legs	Right	Left
208	+	0	+	0	ı	Impalp
258	0	0		0	Normal	
298	0	0		+	Normal	
262	0	0		+	Normal	
314	0	0		+	Normal	
247	0	0		0	Normal	Weak
207	0	0		0	Normal	
249	0	0		0	Normal	
243	0	0		0	Normal	
198	0	0		0	Normal	
278	+	0	+	0	Weak	Weak
188	0	0		0	Normal	Unpalp
226	0	0		0	Normal	
304	0	0		0	Impalp	Impalp
200	+	++		+	Normal	
194	0	0		+	Normal	
216	0	0		0	Normal	
231	0	+		+	Weak	Weak
226	0	0		0	Normal	
203	0	0		0	Normal	
240	0	0		0	Weak	Normal
143	0	0		0	Normal	
153	0	0		0	Normal	
158	0	0		0	Normal	
247	0	0		0	Weak	Normal
215	0	0		0	Normal	
224	0	0		0	Normal	
244	0	0		0	Normal	
167	0	0		0	Normal	
211	+	+++		++	Normal	
143	0	0		0	Normal	

foot and ankle On readmission in April 1965 the residual part of the right foot was red and swollen The dorsum of the foot showed an ulceration with pale granulations in the floor The dorsal artery of the foot could not be palpated The diagnosis of arteriosclerotic gangrene of the foot was made Low amputation of the right lower leg was performed The stump healed well No pain after operation No chest pain

Examination Sept 14 1965 Heart physically normal Low amputation of the right lower leg The dorsal artery of the left foot could not be palpated Roentgen examina

tion showed fine calcification of the aortic arch The abdominal aorta showed no calcification On the other hand calcifications were seen at the site corresponding to the common iliac artery No calcifications were seen in the left thigh or left lower leg

This patient thus had gangrene of right foot, probably due to arteriosclerosis and small calcifications in the aortic arch and the common iliac artery

K M fam 2 III 24 male, born 1911 No chest pain Slight leg pain unrelated to exertion Roentgen examination showed a *few thin calcifications* at the site of the right *femoral artery* Otherwise no arterial calcifications

T M fam 2 III 25 male born 1911 No chest pain or leg pain Roentgen examination showed superficial small mural *calcifications* in the major part of the *femoral artery* The arteries of the lower leg also showed scattered less dense calcifications No aortic calcifications

I A fam 18 II 11 female born 1924 Rheumatic fever at 12 years She is said to have had murmurs afterwards Frequent pain and stitch in the region of the heart not related to physical exertion or to exposure to cold or wind No radiation of the pain She had had pain in the thigh knee and right lower leg unrelated to physical exertion Exercise ECG showed promptly after 5 minutes exertion (600 kgm/min) nothing remarkable Three min after exertion ST j depression and depression of ST segments in II III and aVF about 0 5 mm and in V_7 about 1 5 mm below the isoelectric line ST segments in II arcuate in III aVF and V_7 horizontal ST j-depression in V_5 about 1 mm ST segment then rising Five min after exertion the changes were of the same type as 3 min after exertion though less pronounced Ten minutes after exertion the ECG was normal

In this patient, the symptoms and the ECG after exercise gave reason to suspect coronary heart disease

G P fam 20 III 1 male born 1909 No chest pain No leg pain Roentgen examination showed *small calcifications of the aortic arch* Plain roentgenograms of the abdomen showed a few small *calcifications in small pelvic arteries* No vascular calcifications in the thigh or lower leg

G L fam 25 III 2 female, born 1918 For 10 years she had had a high systolic blood pressure — 175—185 mm Hg Sometimes she had stabbing pain in the region of the heart Shortness of breath and pain in the entire chest on exertion e g when ascending hills or steps The pain was not of radiating type No pain in the legs but pain in the feet unrelated to exertion Heart physically normal B P 175/105—105 ECG ST j depression almost 1 mm below the isoelectric line in I V_2 and V_3 0 5 mm below the isoelectric line in II and V_4 ST segments in these leads were slightly descending R_{V4-V6} 8—12 mm ECG after exercise was not done because of the patient s poor general condition

This patient thus had moderate benign hypertension and coronary heart disease was likely

E N fam 42 II 1, female born 1888 Shortness of breath when walking up hills worse in fog wind and cold No chest pain No leg pain Heart physically normal B P 170 90—80 ECG ST j and ST segments depressed about 0 5 mm in V_4 V_5 and V_6 ST segment there horizontal T waves isoelectric in aVF R peak there about 5—6 mm T waves shallow in V_4 V_5 and V_6 (less than 1/10 of corresponding R waves) Roentgen examination showed insignificant mural calcification of the thoracic aorta The abdominal aorta showed moderate mural calcifications Thin arterial calcifications in both thighs and lower legs

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From Department of Medicine C (Heads N B Krarup M D and B Harvald M D) and Intensive Therapy Unit R (Heads W Dam M D and J Hagelsten M D) Bispebjerg Hospital Copenhagen Denmark

Alkalosis in Patients with Respiratory Insufficiency

By

Bent Harvald Lise Hendriksen and Lene Hoy

The hypercapnia in patients with pulmonary insufficiency tends to lower the blood pH The kidney tries to compensate this respiratory acidosis by production of acid urine with contemporary retention of bicarbonate Thus increase of the carbon dioxide tension (pCO_2) increases the carbonic anhydrase activity in the distal tubules with production of H_2CO_3 from CO_2 and H_2O H_2CO_3 is dissociated into H ions and HCO_3^- ions The former are exchanged with Na ions in the pre urine whereas the HCO_3^- ions are retained By this process it is possible to keep blood pH normal or just slightly acid in spite of considerable pulmonary insufficiency with CO_2 retention

However an apparent overcompensation is frequently seen with a combination of hypercapnia and alkalosis Thus among 156 patients with primary lung disease Robin (5) found arterial blood pH over 7 45 in 21 This cannot be explained by the process mentioned above, which should theoretically be able to bring blood pH towards the initial value only without leading in itself to an overcompensation

Several possible explanations of this paradoxical phenomenon have been discussed (1, 2 4 5) The slow rate of renal bicarbonate excretion is considered most important Improvement of the pulmonary function will lead to an immediate fall in pCO_2 whereas the bicarbonate retained during periods with respiratory acidosis is only excreted slowly thus allowing for the transient appearance of alkalosis Other possible factors of importance are depletion of chloride and potassium which would be able further to delay the renal excretion of bicarbonate Potassium depletion particularly is emphasized as essential for the development of alkalosis whether the hypopotassemia is due to treatment with diuretics or is somehow secondary to the pulmonary disease (1 4)

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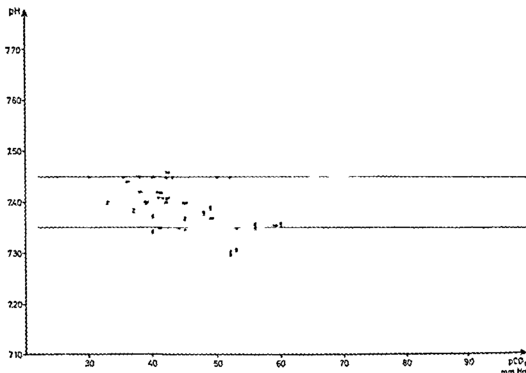


Fig 1 pH in patients with primary lung disease with different degrees of respiratory insufficiency The pCO_2 values have been calculated from standard bicarbonate (oxygenated blood) and pH ✓ = patients under thiazide treatment at the time of examination

Material

Fig 1 gives the results of 189 examinations of arterial blood in 117 patients admitted to Bispebjerg Hospital medical department C during the years 1963—65 because of lung disease with varying degrees of respiratory insufficiency In 16 of these patients pH above 7 45 was found which is nearly the same proportion of the patients as in Robin s series The highest pCO_2 observed with a pH above 7 45 is 62 mm Hg which is a degree of hypercapnia usually combined with respiratory acidosis

Table I gives some of the biochemical details in these patients It is characteristic that the respiratory function of nearly all is either improving or at least stationary at the time of examination In most of the patients the standard bicarbonate is high

In order to elucidate the origin of the alkalosis we have considered the conditions in patients with pulmonary insufficiency during respirator treatment Application of controlled ventilation brings about a sudden improvement of the respiratory function A steep rise in plasma pH might be expected to follow the reduction of pCO_2 At the same time the renal excretion of HCO_3 ions and reabsorption of H ions might be expected to increase resulting in a decrease of the plasma standard bicarbonate The case histories and the biochemical findings in three patients in whom controlled ventilation was applied will be presented in detail (fig 2)

Case reports

Case 1

A 73 year-old former mechanic with bronchitis for about 20 years Admitted in a state of severe pulmonary insufficiency

TABLE I Biochemical findings in 16 patients with primary lung disease and alkalosis An indication is given whether the respiratory function was improving stationary or deteriorating at the time of examination and whether thiazides were given

Sex	Age	Respiratory function	Thiazides	pH	Oxygen saturation (%)	Standard bicarbonate (mMol/l)	pCO_2 (mm Hg)
♀	57	Stationary	−	7 50	90	28 9	39
♀	75	Stationary	−	7 47	78	22 2	32
		Improving	−	7 49	93	24 8	34
		Deteriorating	−	7 50	84	28 0	38
♂	64	Improving	+	7 55	83	30 5	38
		Stationary	−	7 50	95	29 2	40
♂	48	Improving	+	7 46	91	26 2	39
		Improving	+	7 47	95	28 8	42
♀	51	Improving	−	7 47	74	38 0	58
		Deteriorating	+	7 46	56	40 0	62
		Improving	+	7 46	87	32 0	49
		Improving	+	7 47	83	40 0	62
		Stationary	+	7 50	86	33 0	45
♀	69	Improving	−	7 43	92	23 7	33
♂	77	Stationary	−	7 46	86	29 0	42
♀	79	Stationary	+	7 48	92	29 9	43
♂	70	Stationary	−	7 46	68	34 8	54
♂	76	Improving	−	7 46	91	24 0	36
♀	71	Improving	+	7 48	85	28 6	40
♀	65	Improving	+	7 48	86	25 9	37
♂	53	Improving	+	7 47	88	29 0	42
♀	46	Stationary	−	7 53	89	27 5	34
		Stationary	−	7 48	78	31 0	44
		Stationary	−	7 47	83	36 5	37
♂	81	Stationary	−	7 51	92	23 6	31
♂	69	Improving	+	7 55	94	25 8	30
Average					85	29 6	41 5

with dyspnea intense cyanosis mental confusion and muscular twitches He was treated with oxygen via nasal catheter The condition worsened and after a few *hours the patient gradually became comatose* Arterial blood examination showed reduced oxygen saturation high pCO_2 and severe acidosis After previous tracheo-bronchial toilet controlled ventilation was initiated (Bennett s respirator) via oro-endotracheal tube followed by tracheotomy This quickly brought about an improvement of the respiratory condition with elevation of the oxygen saturation to subnormal values and *lowering of pCO_2 Before pCO_2 had regained* normality acidosis had changed into alkalosis (pH 7 56 There was a sudden rise in standard bicarbonate together with a fall in serum potassium The patient s psychical condition improved somewhat but he did

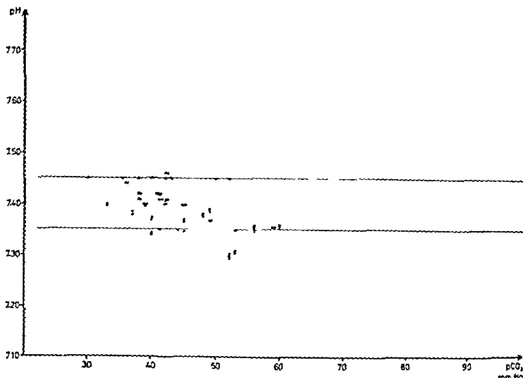


Fig 1 pH in patients with primary lung disease with different degrees of respiratory insufficiency The pCO_2 values have been calculated from standard bicarbonate (oxygenated blood) and pH = patients under thiazide treatment at the time of examination

Material

Fig 1 gives the results of 189 examinations of arterial blood in 117 patients admitted to Bispebjerg Hospital medical department C during the years 1963—65 because of lung disease with varying degrees of respiratory insufficiency In 16 of these patients pH above 7 45 was found which is nearly the same proportion of the patients as in Robin s series The highest pCO_2 observed with a pH above 7 45 is 62 mm Hg which is a degree of hypercapnia usually combined with respiratory acidosis

Table I gives some of the biochemical details in these patients It is characteristic that the respiratory function of nearly all is either improving or at least stationary at the time of examination In most of the patients the standard bicarbonate is high

In order to elucidate the origin of the alkalosis we have considered the conditions in patients with pulmonary insufficiency during respirator treatment Application of controlled ventilation brings about a sudden improvement of the respiratory function A steep rise in plasma pH might be expected to follow the reduction of pCO_2 At the same time the renal excretion of HCO_3 ions and reabsorption of H ions might be expected to increase resulting in a decrease of the plasma standard bicarbonate The case histories and the biochemical findings in three patients in whom controlled ventilation was applied will be presented in detail (fig 2)

Case reports

Case 1

A 73 year-old former mechanic with bronchitis for about 20 years Admitted in a state of severe pulmonary insufficiency

pCO_2 was still over the normal range As at the same time the patient regained consciousness the respirator treatment was discontinued after 12 hours During oxygen administration pCO_2 rose again whereas pH and standard bicarbonate decreased Controlled ventilation was reintroduced leading to the same changes as before It was continued for another 15 hours after which the patient was stabilized During the following period where arterial blood examination was made only occasionally standard bicarbonate and pH slowly approached normal values Seventeen days after admission the patient was discharged in his habitual state

Case 3

A 46-year-old housewife with bronchial asthma for about 10 years During later years increasing abuse of morphia Admitted to the psychiatric ward because of acute psychosis Treatment with large amounts of sedatives during which acute pulmonary insufficiency developed with low arterial oxygen saturation hypercapnia and acidosis As the patient became deeply unconscious controlled ventilation (Bennett s respirator) was initiated after previous tracheo-bronchial toilet followed the next day by tracheotomy The arterial oxygen saturation rose, pCO_2 fell and during the first day the pH was brought from 7 20 up to 7 42 There was a slight rise in standard bicarbonate The patient regained consciousness and from the third day ventilation was assisted only intermittently During the following days pH and standard bicarbonate again fell slowly to a slightly acidotic level. As after one week the patient was still dependent on the respirator she was transferred to a chronic pulmonary insufficiency wa d

Discussion

The sudden improvement of the respiratory function during controlled ventilation in a short period of time brings the patients from acidosis into alkalosis at a time when pCO_2 has certainly fallen but is still over the upper normal limit Thus the paradoxical type of alkalosis has developed Together with the rise in pH, standard bicarbonate concentration increases During the following period with stabilized respiratory function standard bicarbonate as well as pH fall slowly

The course of events induced by the improved ventilation with a rapid change from acidosis to alkalosis followed by a gradual fall of pH and standard bicarbonate clearly demonstrates the validity of Robin s theory (5) according to which the renal excretion of bicarbonate being a more time-consuming process cannot keep pace with the pCO_2 fall which is an immediate consequence of the improved ventilation The initial rise of the standard bicarbonate is difficult to explain but might be due to the elimination of acid metabolic products (lactic acid?) after improved oxidation of the arterial blood In patient 3 there is only a slight rise of the standard bicarbonate, probably because there has been less accumulation of acid products in this patient in whom the arterial oxygen saturation has not been so low

Refsum (4) and Cockran (1) thought hypopotassemia to be of essential importance for the development of the alkalosis In connection with the respirator treatment these 3 patients showed a fall in serum potassium of varying size (4 7—2 0 3 8—3 1 4 3—3 9 mEq/l) probably explained by migration into the cells of potassium from the extra cellular space during the return to normal of the pH This reduction of serum pota ium will of course be

able to impede the renal excretion of HCO_3^--ions and retention of H^+-ions and thereby may influence the time required for the kidney to reestablish a normal pH Low serum chloride exerts a similar effect (3), but cannot possibly be essential for the development of alkalosis in these patients Thus the patient with the highest pH in our group (case 1) had a high serum chloride level

Treatment with chlorothiazide calls forth a metabolic alkalosis thus accentuating the alkalosis of the type described here Marked alkalosis however, was seen also in patients who were not under thiazide treatment (fig 1 and table I)

Even if it has thus been demonstrated that acute improvement of ventilation may give rise to the development of alkalosis in patients with pulmonary insufficiency, this of course is not necessarily always an adequate explanation of alkalosis in patients with impaired pulmonary function Uneven ventilation of different lung sections may give rise to high pH of the arterial blood but in such cases pCO_2 and standard bicarbonate will be lowered The fact that reduced hemoglobin is a stronger base than oxyhemoglobin is probably of minor importance

Summary

In a consecutive series of 117 hospitalized patients with primary lung disease and different degrees of respiratory insufficiency alkalosis was found in 16 In some of these alkalosis was combined with hypercapnia A pCO_2 of 62 mm Hg was the highest recorded with pH above 7 45 The standard bicarbonate was elevated in most of these cases

In patients with improving respiratory function alkalosis may be due to a proportionately slow renal excretion of the bicarbonate retained as a compensatory measure during periods of respiratory acidosis This mechanism is demonstrated in three patients with respiratory insufficiency in whom controlled ventilation is applied The acute improvement of ventilation called forth a pronounced alkalosis at a time when pCO_2 was still over the normal level After an initial rise, probably due to oxidation of accumulated acid metabolic products, standard bicarbonate gradually decreased during the following days and synchronously pH became normal

Acknowledgement

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From the Department of Medicine (Head G Biörck M D) Karolinska Institutet at the Seraphimer Hospital Stockholm Sweden

Clinical Trial with Ethyl-α-p Chlorophenoxyisobutyrate (Atromid S) with Special Reference to White Blood Cell Counts

By

Jorgen Engstrom

In 1962 Thorp reported on the serum lipid reducing activity of Atromid a combination of ethyl α p chlorophenoxyisobutyrate (CPIB) and androsterone in experimental animals (22) In the same year Oliver published the first clinical trial in man (15) In later comparative studies on Atromid and CPIB alone (later called Atromid S) Oliver concluded that both were equally effective in lowering elevated serum cholesterol levels and probably also in reducing elevated triglycerides (17) Later several reports confirmed this and treatment with the combination was abandoned

At the Seraphimer Hospital a clinical trial was started in 1963 and preliminary results were presented in a previous paper 6 *The present paper deals with* the results of the continued trial

Material

Twenty two patients were treated with Atromid or Atromid S Of these patients 3 received treatment for 2 months or less As the effect on serum lipids could not be evaluated in such a short time they were excluded from the trial The reason for discontinuing in these 3 patients was lack of co-operation in 2 and the appearance of a maculo-papular rash which could not with certainty be ascribed to Atromid in the third

The group studied thus consists of 19 patients of whom 12 were female with a mean age of 56 years (44—66) and 7 male with a mean age of 45 years 34—53

Of these 19 patients 16 showed signs of cardiovascular disease 3 were treated solely because of elevated serum lipid levels

Methods

All the subjects were out patients but for a 7-day period before the beginning of treatment when they were hospitalized in the metabolic ward for determination of serum lipid levels liver function tests and in order to exclude biliary disease and hypothyroidism They were instructed to continue with their customary diet without changes during the treatment

The laboratory tests were carried out in the Department of Clinical Chemistry at the Seraphimer Hospital Head R Blomstrand M D Methods and normal ranges are given in table I

Submitted for publication on April 12 1966

TABLE I Methods and normal ranges

Analysis	Method	Unit	Normal range in serum
Cholesterol (to Febr 1964)	Pearson et al (19)	mg/100 ml	150—300
Cholesterol (from Febr 1964)	Autoanalyzer method N 37 Levine & Zak (14)	mg/100 ml	141—284
Triglycerides	Blankenhorn et al modified by Blomstrand (2)	mg/100 ml	50—150
Bilirubin	Jendrassik & Grof (10)	mg/100 ml	<1 0
Glutamic — oxalo — acetic transaminase GOT	Karmen et al (11) modified by Ordell Kabi Co Stockholm	units/ml	10—35
Glutamic — puruvic trans aminase, GPT	Wróblewski & LaDue (25) modified by Ordell Kabi Co Stockholm	units/ml	10—35
Alkaline phosphatase	Bessey et al (1) Modification Sigma Technical Bulletin 104 (3—1963)	units/ml	0 9—2 5
Thymol test	McLagan & Bunn (12)	units/ml	<4 0
Prothrombin — proconvertin value	Owren & Ass (18)		80—120 % of the standard
Protein electrophoresis	Paper electrophoresis according to Laurell et al (13) evaluated by Spinco Analytrol Model RB Beckman Instr Inc USA	g/100 ml	albumin 4 42 (3 48—5 36) α_1 globulin 0 20 (0 19—0 39) α_2 globulin 0 51 (0 29—0 73) β globulin 0 84 (0 52—1 16) γ globulin 1 15 (0 71—1 59)
Bromsulphalein test 5 mg/kg body weight	Rosenthal & White (1925)	retention in 45 min as % of the 5 min value	<5
Statistical evaluation of changes in white cell counts	Wilcoxon test Documenta Geigy Wissenschaftl Tab 6 Aufl		
Statistical evaluation of the relationships between white cell counts and serum lipids	Spearman s rank correlation test Documenta Geigy Wissenschaftl Tab 6 Aufl		

"Pre treatment levels of lipids and blood cell counts

Before the beginning of treatment at least 3 serum cholesterol and 2 serum triglyceride determinations were made In most cases additional results from the year previous to the trial were included when determining the mean pre treatment levels

Patients with mean serum cholesterol levels above 300 mg/100 ml were included in

the trial At the beginning of the trial cholesterol was determined according to Pearson (19) From Febr 1964 onwards automated determinations according to Le Vine and Zak were used (14) Comparative studies showed that the change to automated determinations resulted in a mean reduction in serum cholesterol levels of 12 % (3) Consequently when analyzing the changes during treatment values obtained with the former method were reduced by 12 % resulting in 295 mg/100 ml as the lowest pre treatment level

Before treatment hemoglobin concentration platelets white cell counts and differential counts were done by conventional methods In determining pre treatment levels available values from the preceeding year were included

Liver function tests before treatment included serum bilirubin SGOT, SGPT alkaline phosphatase thymol turbidity prothrombin proconvertin concentration and protein electrophoresis In several cases bromsulphalein loading tests were performed

Hypothyroidism was excluded on clinical grounds in combination with a normal BMR and protein bound iodine

Treatment was instituted with Atromid or Atromid S 0 75—1 0 g daily during the first month and in most cases the dosage was increased after varying intervals to a maximum of 2 0 g daily In May 1964 Atromid S was substituted for Atromid Only three subjects were given Atromid during the first 3—5 months of the trial

During the trial the patients were controlled at pre-determined intervals of maximally 3 months In the beginning of the study only serum lipids and liver function tests were followed Since Prout and Edwards reported a case of agranulocytosis during Atromid treatment (20) the following tests were done at every visit hemoglobin concentration platelets white cell count total and differential SGPT prothrombin proconvertin concentration cholesterol and triglycerides

The following additional tests were performed on several patients at different intervals serum protein electrophoresis bromsulphalein tests and sternal marrow puncture

Treatment levels were defined as follows

for cholesterol and triglycerides respectively the mean values from the last two visits during treatment

for hemoglobin concentration platelets white cell count, polynuclear white cells mononuclear white cells and SGPT respectively the mean values of the determinations made two months or later from the beginning of treatment

The reason for omission of determinations made during the first two months of treatment was to avoid changes secondary to the initial shift in lipid metabolism If a patient was examined between the pre determined visits i e because of increasing angina myocardial failure of infections the values obtained at such occasions were excluded in order to avoid periods when the patient was out of balance

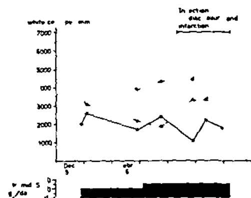
Case reports

In the following case reports of the 3 patients who died during the trial will be given and of one patient with marked leukopenia

Case 3 (fig 1)

Woman aged 61 with angina pectoris since 1960 when xanthomatosis was noticed Pneumonia 1961 Leukopenia had not been observed before the trial During Atromid S treatment there was a decrease in white cell counts but not to pathological levels After 3 1/2 months she suffered recurrent respiratory infection She was hospitalized after about 4 months of treatment for left ventricular failure bronchopneumonia and pleural effusion In connection with these complications white cell counts and polynuclear white cells increased

She was again admitted to hospital after 5 months of treatment with more frequent attacks of angina pectoris increased myocardial insufficiency and pleural effusion Hb concentration was 11 0 g 100 ml compared with 12 7 before the trial the


F g 1 Wh e cell coun s (case 3) To al wh e cell count (● — —●) polymorphonuclear wh e cells (● — ● mononuclear h te cells (●———●)

w hite cell count as st ll normal 2 000 ml of blood sta ned flu d as asp rated by thora cocentes s Af er 10 days she d ed from ventricular f br llat on

A post mortem exam nat on sho ed a poster or myocard al nfarct on aged 3 to 5 weeks septal nfarct on aged abo t 2 eeks as well as a recent nfarct on in ol ing the anter or all

In the femoral d aphys s there as red bone marro h ch on m croscopy was found to be hematopoet c to an extent of about 40 The cell compos t on was no mal but the extens on of red bone marro as ren arkable and could scarcely be a compensator pl enomenon following the blood loss n the pleural effus on

Ca e 4 (fig 2)

Woman aged 56 without a h story of leukopen a D fferent al counts were normal t ice w th n the 4 months preceed ng treat ment This pat ent was treated th Atrom d during 5 months thereafter w th Atrom d S She as one of the two pat ents in w hom the hite cell counts were not regularl con trolled dur ng the earl er part of the trial After 5 months of treatment a total wh te cell count mononuclear and pol nuclear w h e cells sl o ed lo v values As th s leukopen a was st ll observed 2 months later Atrom d S was d scontinued During tl e follo ng 3 weeks w h te cell counts were normal on 3 occas ons Af er 7 weeks and 2 mon hs leukopen a recurred

The sternal marrow af er 2 months of d scontinued treatment showed s gns of s deropen a sl ghtly hyperplast c myelopoesis and a certa n degree of tox c granula t on Three months earl er her serum iron had been 72 μg 100 ml w l ch led to iron subst tut on Hb concentrat on at the t me of sternal puncture as 11 0 g 100 ml

After 3 months the drug as re nst u ed at a decreased dosa e 1 0 g da ly A week later leukopen a was aga n no ced (2 400 mm³ polynuclear h te cells 90 and Atrom d S was aga n d scont nued

The w h te cell co nt rema ned below 4000 mm³ during tl e follo ng 4 mon hs During tl e next 10 months normal wh e cell co unts includ ng d fferent al coun s were found on 3 occas ons


F g 2 Wh e cell counts (case 4 To al wh e cell count (● — ●) polymorphonu l ar wh e cells (● ●) mononuclear wh e cells ●———●)

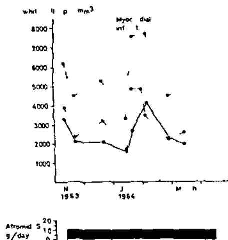


Fig 3 White cell counts (case 7) Total white cell count (●— — —●) polymorphonuclear white cells (●- - -●) mononuclear white cells (●———●)

In this case there was possibly a spontaneous tendency to leukopenia accentuated during Atromid S treatment

Case 7 (fig 3)

Woman aged 59 with myocardial infarction about 6 months before the trial A low white cell count was observed after 2 months of treatment after which values again returned to normal Despite a low dosage serum cholesterol and triglycerides became almost normal She died from myocardial infarction after 5 months of treatment At post mortem examination healed myocardial infarctions were found as well as infarctions aged 3 to 8 weeks in addition to a fresh infarction On microscopy the vertebral marrow showed fatty marrow to an extent of about 60 % with ordinary cellular composition in the hematopoetic part (fig 6) There was swelling of the reticulum and reduction of the lymphoid apparatus in the spleen (fig 7) Venous congestion was seen in the liver with a degree of fatty degeneration

Case 12 (fig 4)

Woman aged 64 with angina pectoris since 1944 and a history of myocardial infarction on 4 occasions since 1948 She also had intermittent claudication Treatment with Atromid S had moderate effect on her serum lipids. The white cell counts successively *decreased during the last 7 months of treatment* Atromid S was discontinued after one year because of abdominal distress and nausea About 2 weeks later she died within 24 hours from the onset of myocardial infarction

On the day of infarction she had low white cell count of 3 100/mm³ At post mortem examination there were healed myocardial infarctions as well as a recent infarction The liver was normal macroscopically as was the spleen On microscopy the vertebral marrow showed fatty marrow to an extent of about 40 % with ordinary cellular composition in the hematopoetic part (fig 8)

This increase in fatty bone marrow was evaluated as pathological

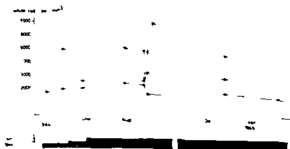


Fig 4 White cell counts (case 12) Total white cell count (● — — ●) polymorphonuclear white cells (●- - -●) mononuclear white cells (●———●)

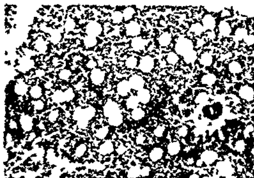


Fig 5 Normal vertebral marrow for comparison van Gieson × 50



Fig 7 Reduction of the lymphoid apparatus in the spleen and swelling of the reticulum (case 7) van Gieson × 50



Fig 6 Fatty infiltration in the vertebral marrow (case 7) van Gieson × 50

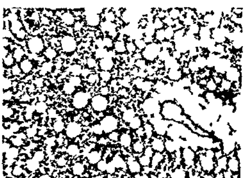


Fig 8 Fatty infiltration in the vertebral marrow (case 12) van Gieson × 50

Results

Duration of treatment mean dosage and causes of discontinuation

The patients are listed in table II

At the time of this analysis the mean duration of treatment was 13 months (2—27) Ten patients had been given Atromid or Atromid S for more than a year and 12 were still receiving treatment Treatment was stopped earlier than planned in 7 patients Three patients died from myocardial infarction Leukopenia was the reason for discontinuation in one and lack of co-operation the reason in the remaining three patients

The mean dosage at the last two visits was 1 53 g daily (1 25—2 0)

Clinical symptoms

Fifteen patients in the group showed electrocardiographical signs of ischemic heart disease, and 14 patients had attacks from angina at the beginning of treatment Of those, 6 subjects experienced less frequent distress during the trial while 8 patients were unable to notice

TABLE II Serum cholesterol and triglycerides during treatment with Atromid S

Case	Age	Sex	Months of treatment	Mean dosage at the last two examinations (g/d)	Pre treatment: Cholesterol (mg/100 ml)	Pre treatment: Triglycerides (mg/100 ml)	Treatment: Cholesterol (mg/100 ml)	Treatment: Triglycerides (mg/100 ml)	Cholesterol reduction as % of pre treatment level	Triglycerides decreased as % of pre treatment level
1	55	♀	14	1 3	351	159	357	135	2	15
2	50	♂	25	1 25	398	875	305	218	23	65
3	61	♀	5	1 25	465	213	503	220	− 9	− 3
4	55	♀	7	1 5	371	205	301	126	19	39
5	66	♀	17	1 25	389	438	337	185	13	58
6	52	♂	23	1 75	295	460	280	161	5	64
7	59	♀	5	1 0	348	223	298	153	12	31
8	49	♀	24	1 5	507	156	416	134	18	14
9	53	♂	27	2 0	487	1 487	415	752	14	49
10	40	♂	11	1 5	359	152	353	183	2	20
11	64	♀	18	1 5	468	316	371	181	21	43
12	64	[illegible]	12	1 5	48	252	40?	214	27	15
13	45	♂	8	2 0	341	312	296	244	13	22
14	47	♀	2	2 0	389	331	378	248	3	25
15	60	♀	14	1 5	345	1 100	604	795	75	27
16	50	♀	1	1 5	306	2 520	243	177	21	93
17	44		8	1 75	457	379	450	219	2	40
18	58	♂	7	1 5	4 4	299	378	188	29	37
19	34	♂	7	1 5	458	146	369	123	19	16
Mean values			13	1 5	407	523	369	245		

any change Three female patients died from myocardial infarction two after five months and the third after a year A male patient (case 18) had a recurrence of myocardial infarction after 7 months of treatment followed by a post myocardial infarction syndrome from which he recovered

Five subjects had signs of obliterative arterial disease of the lower limbs A female patient (case 15 had previously been operated on with the construction of a by pass and was free from symptoms at the beginning of the trial Four patients had intermittent claudication In a female patient with hyperlipemia (case 16) walking capacity increased from 500 meters to several kilometers In a male patient with hyperlipemia arteriography had shown multiple stenosing changes in both legs inaccessible to surgery case 9) He noticed improve

ment during the trial After one year his condition was complicated by a total occlusion in the left femoral artery, verified by arteriography Apart from this the extensive atheromatous plaques in both legs and external iliac arteries were unchanged Some weeks later there was gangrene of the foot, and amputation was carried out after 15 months of Atromid treatment

The regression of intermittent claudication in a female patient (case 12) was probably due to restricted exercise because of heart failure, which developed during the trial She died later from myocardial infarction

A female patient (case 17) had slight bilateral intermittent claudication before the trial After two days of treatment with Atromid S, 1 0 g daily, the right femoral artery was catheterized when performing coronary angiography Three days later the patient showed symptoms of occlusion of the right external iliac artery and oscillometry suggested total occlusion Treatment with streptokinase was followed within 24 hours by complete restitution of the circulation (4 After this episode the patient was treated with Atromid S for 8 months and was free from claudication

Changes of serum lipid levels

Serum cholesterol and triglycerides are reported in table II

Serum triglycerides

The mean pre treatment triglyceride level was 528 mg/100 ml (146—2 520), mean treatment level was 245 mg 100 ml (123—795) A reduction exceeding 10 % of the pre treatment value was seen in 17 of the 19 subjects, and in these the mean reduction was 38 % (14—93) In 2 patients the level increased during treatment, by 3 and 20 % respectively

Serum cholesterol

Mean serum cholesterol before treatment was 407 mg/100 ml (295—548) and the mean treatment level 369 mg/100 ml (243—604) In 12 out of the 19 patients the reduction exceeded 10 % of the pre treatment level, and in these the mean reduction was 19 % (12—29)

In 4 patients there was reduction of less than 10 %

In three cases the treatment level was higher than the pre treatment level, exceeding it by 2, 9 and 75 % respectively The last patient (case 15) had marked hyperlipemia and diabetes treated by diet and chlorpropamide for the last 4 years During the first 5 months of Atromid S treatment serum cholesterol was about the same level as before treatment, 340 mg/100 ml, but serum triglycerides were reduced from 1100 to 277 mg/100 ml During a temporary divergence from her prescribed diet she gained 6 kg in weight Simultaneously there was a rise in cholesterol and triglycerides to 601 and 2 569 mg/100 ml respectively At the last visit a reduction, to 304 and 721 mg/100 ml respectively, was again observed

It should be emphasized that the calculation of the serum lipid changes includes values from those 7 patients in whom treatment was discontinued, before maximal dosage could be tried

Liver function tests

BSP

Bromsulphalein test with 5 mg BSP per kg body weight was done before treatment in 12 subjects (fig 9)

In 9 patients the retention after 45 minutes was 5 % or more of the 5 minutes value This test was repeated after various intervals during treatment in 6 of these 9 patients, and retention values changed to below 5 % in 5 cases One of the patients (case 3) who initially had a pathological retention of 11 %, showed 8 % after a month The test was not repeated, as the patient died from myocardial infarction

Two of the 3 patients who originally had normal retention values continued to have these after one and 12 months respectively the third patient (case 2) showed an increase after 6 months to 8 6 % compared with 3 5 % before the trial Signs of liver dysfunction had previously been observed in this patient, as BSP retention value of 16 % and congestive changes in a liver biopsy specimen had been found 18 months before the trial

The tendency towards normalisation of the retention values could be due to accelerated excretion as well as to increased storage capacity of the liver

S GPT

The treatment level of S GPT was below 35 units in 17 cases and borderline in 2 patients Case 2 as was mentioned above had previously shown signs of liver disease and treatment level was 39 units In case 10 the pre treatment level was 38 units No change was

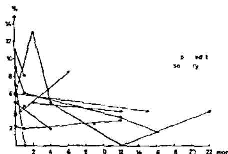


Fig 9 Bromsulphalein test (5 mg/kg body weight) Retention after 45 min as a percentage of the 5 min value

observed during the trial and in this patient alcoholic liver damage was the probable explanation

In case 9 there was a tendency towards improvement in S GPT (from 46 to 30 units) as well as in BSP retention (6 % before treatment 0 to 4 % after one and two years respectively)

Prothrombin proconvertin index

The prothrombin proconvertin values did not show any remarkable changes during the trial, in patients not treated with anticoagulants Four patients on dicumarol continued treatment with this drug during the trial and bleeding episodes were not noticed Following the report by Oliver et al (16) in which a reduction in the requirement of anticoagulants during Atromid treatment was pointed out a reduction of dicumarol dosage together with frequent controls was employed during the first month and in connection with increase of Atromid S dosage

Serum protein electrophoresis

Protein electrophoresis was done before and at various stages during treatment

TABLE III White cells per mm³ before and during treatment

Case	Pre treatment level			Treatment level		
	Total white cells	Polymorpho-nuclears	Mono-nuclears	Total white cells	Polymorpho-nuclears	Mono-nuclears
1	6 200	3 400	2 800	5 130	2 830	2,300
2	4 000	2 600	1 400	3 740	1 940	1,800
3	5,700	3 700	2 000	4 150	2,750	1 400
4	6 800	4 100	2 700	3 600	2 000	1 600
5	4 500	2 200	2 300	3 620	1 680	1 930
6	8 300	5 700	2 600	5 450	3 720	1 730
7	6 200	3 900	2 300	3 950	2,150	1 800
8	4 200	2 700	1 500	4 410	2 780	1 640
9	4 300	2 800	1 500	5 790	3 680	2 110
10	6 300	4 100	2 200	4 200	2 600	1 600
11	7 600	4 200	3 400	6 140	3 480	2 660
12	6 200	3 400	2 800	4 430	2 580	1,850
13	8 600	5 500	3 100	6 700	3 900	2,800
14	5 100	3 800	1 300	5 400	3 800	1 600
15	4 500	3 000	1 500	4 970	3 170	1 800
17	5 700	4 000	1 700	5 500	3,700	1 800
18	10 000	7 250	2 800	6 200	4 100	2 100
19	5 000	2 400	2 600	5 300	3 400	1 900
Mean	6 067	3 817	2 250	4 927	3 014	1 912
Range	4 000–10 000	2 200–7,200	1 300–3 400	3 600–6 700	1 680–4 100	1 400–2 800

in 14 patients No significant changes occurred

Thus hepatotoxic effects from Atromid S were not revealed by the liver function tests performed

Hematology

Hemoglobin concentrations and platelet counts did not change during the trial The mean pre treatment hemoglobin was 13 0 g/100 ml (11 2—14 2), the mean treatment level 12 5 (11 1—14 0) Corresponding platelet levels were 180,000/mm³ (125—305 000) and 209,000 (109,000—302 000)

Pre treatment and treatment levels of white cell counts are seen in table III (Case 16 was omitted because a differential count had not been performed before treatment)

It is remarkable that 6 out of 18 patients had mononuclear white cells below 2,000 before treatment The polynuclear and total white cell counts were above 2 000 and 4 000 mm³ respectively in all cases

The treatment level of mononuclear white cells was below 2 000 in 13 of the 18 subjects The changes were not significant ($P > 0.10$)

On Dec 20 1958, the patient was admitted to hospital because of a provisional diagnosis of right crural thrombosis and one week's history of swelling and tenderness of the right lower leg and ankle She had first thought that she had wrenched her foot although she could not remember any trauma On admission two-thirds of the right lower leg particularly over the ankle was cyanotic and oedematous The left leg was normal ESR 60 mm/1 hour Body temperature was normal The patient was given dicumarol and heparin and the symptoms soon disappeared She left hospital on Dec 23 She was seen again on Jan 8 1959 by which time all symptoms and signs had disappeared except an increased ESR which was 43 mm/1 hour

This patient thus had slight systolic hypertension, symptoms and ECG suggestive of coronary heart disease, calcifications of the aorta suggesting atheromatosis and a history of suspect thrombosis of right leg which might have been intramuscular bleeding instead

H N fam 42 II 4 female born 1899 Slight pain in the region of the heart unrelated to exertion Shortness of breath when walking up hills or up stairs especially when carrying anything Leg pain unrelated to exertion Roentgen examination showed *thin arterial calcifications in the thigh* and thin arterial calcifications in the *lower leg* No aortic calcifications

I A fam 42 II 7 male born 1966 On a few occasions he had had stabbing pain in the chest unrelated to physical exertion No leg pain Roentgen examination showed *small mural calcifications in the abdominal aorta Calcifications of the media* were seen in both *legs*

S P fam 42 II 9 female born 1912 Slight stitch on left side of the chest on exertion and when hurried No leg pain B P 140/110—100 Resting ECG and ECG after exercise on cycle ergometer (6 min 600 kgm/min) normal

M R fam 42 III 3 female born 1917 The patient had had a feeling of cramps in the region of the heart in association with trouble and unrest some years previously but not on exertion on exposure to cold or when hurried Pain and swelling of the left lower leg unrelated to physical exertion (varicose veins) Heart physically normal B P 150/110—100 ECG T_{V2-V3} neg T_{aVF} isoelectric T_{V4-V6} isoelectric diphasic ST isoelectric

This patient thus had slight ECG changes that might indicate coronary heart disease but the deviations were too small to warrant any diagnosis

A E fam 49 IV 1 female born 1913 Occasional chest pain radiating to left arm when walking up hills or rapidly on level ground especially on acceleration not particularly on exposure to cold No leg pain Heart physically normal B P 145/110—100 Resting ECG normal ECG immediately after exercise on cycle (6 min 600 kgm/min) slight depression less than 0 5 mm of ST-segment in II and aVF ST segments there horizontal or somewhat descending Five minutes after exertion ST segments sloping to between 0 5 and 0 9 mm below isoelectric line in II ST j-depression in V_3 and V_4 about 1 mm in V_5 almost 1 mm below the isoelectric line ST segments in these leads horizontal or somewhat descending

This patient thus had chest pain and exercise ECG typical for coronary heart disease

N F fam 81 female born 1902 For a few months the patient had had left sided chest pain on exertion e g when walking quickly Shortness of breath when walking up hills or stairs Legs often felt cold Once she had severe cramp of the left leg which lasted for

several hours Otherwise no leg pain Heart silent sounds B P 250/150—140 Resting ECG ST arcuately depressed between 0 5 and 0 9 mm in V_2—V_4 T isoelectric in aVF R_{V4-V6} 14—15 mm Immediately after exertion on cycle for 6 min (600 kgm/min) ST j-depression 0 5—0 9 mm below isoelectric line in I and II about 1 mm in V_2—V_4 and CR_{IV} ST segments in these leads were horizontal or descending ST elevation 0 5—0 9 mm above isoelectric line in aVF ST segments there horizontal Five minutes after exertion ST j depression in I and II 0 5—0 9 mm below isoeletric line ST segments there descending Roentgen examination slight calcifications in the aortic arch and marked calcifications in the abdominal aorta Clear-cut calcifications in the thighs Ophthalmologic examination showed moderately sclerotic vessels

This patient thus *had* hypertension, general arteriosclerosis with calcifications in the aorta and pathological exercise ECG suggestive of coronary heart disease Sclerosis of retinal vessels

In 13 cases the ocular fundi were examined by an ophthalmologist In only one patient (N F fam 81) were arteriosclerotic changes observed and were judged as moderate In the remaining 12, and in 19 cases where the eyes were examined by the authors (Silwer and Cronberg) no vascular changes were seen

None of the patients had glucosuria and only one had traces of protein in the urine, but this patient (B J fam 14 II 4) showed no signs of arteriosclerosis In only two of the patients did the serum cholesterol exceed 300 mg 100 ml

Discussion

Necropsy of 3 patients with von Willebrand s disease showed atheromatous vascular changes One of the subjects (F S fam 41) had *not only severe coronary sclerosis* but also pronounced aortic atheromatosis and severe cerebral arteriosclerosis This patient also had developed a fresh thrombus in a coronary artery A second patient (G V fam 42 II 2) had severe arteriosclerotic changes *in the* coronary vessels as well as some atheromatous plaques in the aortic arch The third (N A fam 42 II 8) showed minute atheromatous nodules

Of 31 patients above 40 years with von Willebrand's disease and still living 14 had symptoms that might indicate arteriosclerosis One of them (N F fam 81) had both symptoms and signs of coronary sclerosis and calcifications in the abdominal aorta typical for atheromatosis Two patients (A E fam 49 IV 1 and G L fam 25 III 2) had distinct signs of coronary disease, but roentgen examination revealed no aortic calcifications A further 3 patients (E N fam 42 II 1, I A fam 4 II 7 and W M fam 2 III 15) had more or less pronounced calcifications suggesting atherosclerosis of the aorta or common iliac artery, but no definite signs of coronary sclerosis A calcification in the aortic arch, which was noted in 4 patients is not necessarily a sign of arteriosclerosis for it can also be caused by degenerative changes at the site of the previous orifice of ductus arteriosus *Botalli*

Calcifications in the vessels of the legs were seen in altogether 7 patients including 4 with other signs of arterio-

sclerosis and probably indicated medial arteriosclerosis The patient with circulatory insufficiency and gangrene in the right foot must also be assigned to this group The pathogenesis of medial sclerosis may differ from atheromatosis and is less likely to be influenced by the haemostatic mechanism

The investigation thus clearly showed that arteriosclerosis in the form of atheromatosis and coronary sclerosis is not uncommon in patients with von Willebrand s disease Thus either the arteriosclerotic changes found in these patients have an origin different from that suggested by Duguid or the lack of the von Willebrand factor is unable to prevent the development of mural arterial thrombi It is generally agreed that platelet aggregation and platelet adhesion play a significant role in the development of arterial thrombi (19 20) and in recent years the possibility of defective platelet adhesiveness in von Willebrand s disease has been discussed Thus Borchgrevink (3) and Jorgensen and Borchgrevink (12) have reported a defective platelet adhesiveness in vivo while Ödegaard et al (26) and Salzman (21) have found a defect also in vitro On using Hellem s methods for measuring platelet adhesiveness to glass in citrated whole blood and plasma in 68 patients with von Willebrand s disease we however found no abnormal adhesiveness (6) In the present study one patient who died of myocardial infarction at necropsy was found to have a fresh thrombus in a coronary artery In only one case (E N fam 42 II 1) was there possible but not conclusive evidence of venous thrombosis The possibility thus exists that mural arterial thrombi can develop also in von Willebrand s disease

To decide whether the mechanism of haemostasis, especially the initial phase, has anything to do with atheromatosis and coronary sclerosis, a similar investigation should be carried out on patients with thrombasthenia

Summary

Post mortem examination of 3 subjects above 40 years with von Willebrand s disease revealed that two of them had died of myocardial infarction severe atherosclerotic changes were observed in the coronary arteries as well as in other arteries, and in one of the patients a fresh thrombus was found in a coronary artery Among 31 living patients above 40 years with von Willebrand s disease several cases with coronary heart disease and calcifications in the aorta were seen von Willebrand s disease can therefore not prevent the development of atherosclerosis

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From the Institute for Medical Cell Research, Karolinska Institutet Stockholm Sweden
and The Children's Cancer Research Foundation Boston Mass USA

Cytochemical Population Analyses of the DNA, RNA and Protein Content of Human Leukemic Cells

By

G Gahrton and G E Foley[1]

Previous studies utilizing the biophysical methods developed for the cytochemical analysis of populations of cells (6—10) have indicated that populations derived from neoplastic tissue are characterized by a marked variability in the amounts of cytoplasmic RNA and cytoplasmic protein per cell as compared to populations of cells derived from normal tissue (6 7 25 26) Other studies (17) utilizing these methods of cytochemical population analysis similarly indicated that the amounts of nucleotides and proteins as well as the intercellular variation in nucleotide and protein content per cell in populations of lymphoma cells derived from AK4 leukemia are increased as compared to populations of normal murine lymphoid cells

In a related study undertaken subsequent to initiation of the present investigations these methods of cytochemical analysis have been employed in the study of continuous cultures of human lymphoblasts derived from suspension cultures of the peripheral blood buffy coat of a patient with acute lymphoblastic leukemia (36) These methods thus far have not been applied to the cytochemical evaluation of human leukemic cells derived directly from the patient The purpose of the present report is to describe the results of such cytochemical analyses of populations of cells derived directly from the peripheral blood buffy coats of patients with acute and chronic leukemia with particular reference to the nucleotide content and dry mass (protein content) per cell as well as the intercellular variability of these cytochemical parameters

Material and methods

Material

Since morphologic distinction between polymorphonuclear leukocytes and some blast cells is difficult or impossible in the biophysical instruments used patients with a

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TABLE I. Feulgen DNA, total UV extinction at 265 mμ ($E_{tot}265$) and mass in normal and leukemic cells from the peripheral blood

Case	Diagnosis	Age	WBC × 1,000	Leukemic cells (blood %)	Feulgen DNA Mean relative units	Total UV extinction at 265 mμ: Mean relative units	Total UV extinction at 265 mμ: Coefficient of variation	Mass: Mean relative units	Mass: Coefficient of variation	Treatment
A.W	ALL	7	92	100	1.13	25.9	21.2	7.1	23.1	X ray base of skull, prednisone methotrexate 6 weeks
M.B	ALL	5	957	99	1.25	16.0	19.3	4.0	23.7	None
M.C.	ALL	4	44	93	1.12	22.8	15.9	3.9	31.0	None
D.H	AUL	11	227	99	1.20	20.9	21.9	3.9	24.4	None
J.D	AUL	5	45	80	1.04	23.0	32.4	5.2	(46.6)	None
M.J	AML	4	245	100	1.43	30.2	24.9	4.6	27.3	Methotrexate prednisone cytoxan, vincristine, 11 months. None last 2 weeks prior to present study
N.S	AML	31	400	96	0.76	39.7	26.4	6.7	19.6	None
D.T	AML	12	180	95		26.9	16.7	4.8	24.6	6-mercaptopurine methotrexate prednisone vincristine 9 months
G.D	CLL	66	113	100	1.13	14.8	15.4	3.3	14.8	None
A.C	CLL	80	160	95	1.13	15.4	18.9	2.9	(46.6)	None
Normal human blood lymphocytes					1.00	13.9	10.9	2.5	13.0	
Normal human blood polymorphonuclear leukocytes					0.99	21.1	8.9	6.0	12.8	
Normal mouse lymphocytes from lymph nodes					1.04	14.3	14.9	1.6	16.1	

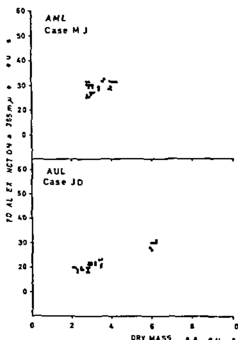
high white blood cell (WBC) count and a high percentage of blasts and/or lymphocytes were selected in order to avoid the necessity of such morphologic identification. Cell populations derived from 8 patients, 4—31 yrs of age with acute leukemia (AL) and 2 patients, 66 and 80 yrs of age with chronic lymphocytic leukemia (CLL), were studied. The acute leukemias were grouped as described elsewhere (32, 47) as acute lymphatic leukemia (ALL), acute myeloid leukemia (AML) and acute unclassified leukemia (AUL). The WBC and percentage of blast cells and/or lymphocytes in the peripheral blood specimens derived from these patients are tabulated in table I. Seven of these patients were untreated and three patients had been treated with various therapeutic agents prior to initiation of these studies.

Isola on of cells and p epa at on of pe nens

About 10 ml of venous blood \ as drawn by cub tal puncture nto an EDTA Vacuta ner (B D Columbus Nebraska USA) con ta n ng 12 mg EDT\ and the tubes imme d ately placed in crushed ice The ent re solat on procedure (15) was then done at + 4 C The blood was mixed w th a solut on of 6 % bo ne fibrinogen (S gma Chemical Company St Lou s M ssouri USA) in sal ne and placed at 60 degrees to the hor zontal for 30 m n to allow the red blood cells to settle The WBC-rich superna tant \ as remo ed and centrifuged at 300 g for 7 m n The supernatant \ as d scarded and the pellet ashed once in 5 ml of 5 % dextrose in d st lled water conta ning 1 mg EDTA per ml Two ml of sal ne \ as added to the ell suspended pellet and 6 ml of d st lled water a then added during gorous shak ng for 30 sec to lyse the rema n ng red blood cells Iso on c ty was restored by adding 2 ml of 3 6 % sal ne and 1 m crol ter of \ar dase solut on (Streptok nase Streptodornase Varidase Lederle Pearl Ri er N Y USA) to prevent clump ng of the WBC The suspens on was aga n centr fuged the supernatant d scarded and the pellet re suspended in a few drops of sal ne Each suspens on as then spread carefully on a ser es of quartz sl des and immed ately f xed b freeze subst u on n l qu d propane held at —196 C n a l qu d n trogen ba h follo ed b immers on n absolute ethanol held at —70 C for 48 hrs in an alcohol CO_2 bath h ch as then allo ed to equ l brate to room temperature (+ 20 C) The prepara t ons ere s ored in fresh absolute ethanol unt l anal zed at vh ch t me they were passed hrough graded absolu e) alcohols to d s lled water One set of sl des \ as then passed o gl cerin and mounted in red st lled glycerin under quartz co ersl ps and sealed h paraff n Dupl ca e sl des were sta ned by the Feulgen react on and mounted in DePe\ G u r London

C opho om y

C topho ome r c meas rements were done n the h gl resolu on rap d scann ng m cro-



F g 1 Total ex nct on at 265 mμ (E 265) n nd dual blast cells plo ted aga nst he mass values of the same cells n populat ons f om a case of AML (M J correla on coeffi c ent 0 82) and a case of AUL (J D correla t on coeffic ent 0 9?) (see text)

spectrophotometer and the h gh resolut on rap d scann ng m cro nterferometer described else here (6—10 33 34) on 100 or more randomly selected cells in each populat on

The D\A content of nd dual Feulgen sta ned cells \ as determined at 546 mμ The mean D\A content of normal control lymphocytes sta ned at the same t me as the patholo cal spec mens as termed 1 rela t e un t RU and all o her determ nat ons ere referred to this alue Cells h D\A values of c rca 1 RU were referred to as 2c cells c be ng the haplo d D\A content

The total nucleo de content of nd dual unsta ned cells as determ ned a 265 mμ The non spec fic absorp on at 315 mμ (E_0 315 as n he range of 10 % of he 265 m absorpt on per ell E 65 Deter m na on of mass and E 65 n the same cells resul ed n orrelat on oeff c en s of 0 82 and

TABLE II RNA DNA rat o n norn al and leukem c h e blood cells

Case	D agnos s	RNA DNA ra o Uncorrected	Correc ed a	Co rrected b
A W	ALL	0 65	0 64	0 59
M B	ALL	<0 10	<0 10	0 10
M C	AML	0 46	0 85	0 81
D H	AUL	0 25	0 38	0 10
J D	AUL	0 59	0 59	0 ?7
M J	AML	0 5?	1 00	0 91
N S	AML	(2 70)	(3 00)	(1 96)
G D	CLL	<0 10	<0 10	0 10
A C	CLL	0 10	<0 10	0 10
Normal human lymphocy es		<0 10	<0 10	0 10
Nort al human polymorpl o clear e koc es		0 53	0 36	0 ?9

Accor l ng to me hods a and b as descr bed by K llander (26)

0 9? respect el n preparat ons der ved from the d fferent pat ents (f g 1) These correla on coeff c ents ind ca e a cons derable degree of constancy n the nucle c ac d concentrat on n nd d al cells of the same populat on It was tl us ass ned that the E 265 alue vas a good measure of the rela e amount of total n cleot des n tl ese cells as has been observed n s m lar stud es th other k nds of cells 6 7 17 25 26

An es mate of the mean relat e RNA content as obta ned by determ nat on of tl e non DNA total ext nct on at 265 mμ (26) Tl e mean DNA content of normal mouse lymphocytes conta n ng negl g ble amo nts of RNA c rca 10 % of the to al nucleot de content has been sl o n to be 14—15 relat ve LA un s 17 26 An approx mately s m lar amount of DNA as found n normal human lym hocytes as in mouse lymphocytes table I The E_{to} 265 value attr butable to DNA could therefore be est mated by detern n ng the Feulgen DNA content and the total E_{ot} 265 on the same cells us ng normal lyn pl ocytes as reference controls for the Feulgen reac t on The E ?65 value attr butable to DNA was then subtracted from the tctal E_o ?65 to obta n the non DNA to al ext nct on at 265 mμ as an est ma e of the RNA content The uncorrected RNA DNA rat o was calculated as the rat of mean non DNA total ext nc on at ?65 m mean DNA total ext nct on at 265 mμ (table II A some l at more accura e es mate of tl e RNA DNA rat o could be ol ta ned f the E 265 values ere corrected fo non spec f c absorpt on at 31 m (4 5 39 as ell as the absorpt d e to pro e n (26) These correct ons (table II) l a e been made accord ng to K ll ler (6 and the true RNA DNA rat o probably l es between these t o corrected val es

The dry mass (referred to as mass of ind v dual cells was d term ned in tl e m cro nterferometer It as a n ed that mass is an approx ma e measure of the prote n content of tl e cell s nce t is kn wn that prote ns const t te 80—90 % of the cell mass (5 ?6)

Results

DNA

The mean DNA content per cell in tl e AL cell populat ons was somewhat larger than the mean DNA content per

cell in normal lymphocyte and polymorphonuclear leukocyte populations (table I, figs 2 3) The basic Feulgen DNA value (42) per cell appears to be circa 10 % larger in most leukemic cells, thus the assumed 2 c value for leukemic cells was circa 10 % greater than that of mature normal lymphocytes and polymorphonuclear leukocytes Cells with DNA values intermediate between 2c and 4c cells were present in most of the AL cell populations 4c cells were present in 4 such populations and 8c cells were present in the populations derived from one case (M J, fig 3) Numerically, the frequency of such cells was consistently low and variable, and appeared to be characteristic of individual cell populations, rather than of a certain AL group The cell population derived from one case of AML (N S, table II) was characterized by a mean DNA value per cell which was significantly (26 %) lower than that characteristic of populations of normal lymphocytes

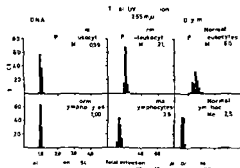


Fig 2 Frequency distribution of DNA $E_{tot}265$ and dry mass per cell in a population of normal lymphocytes and a population of normal polymorphonuclear (PMN) leukocytes

The cell populations derived from two cases of CLL appeared to contain only 2c-cells and in both instances the basic Feulgen DNA values were circa 10 % greater than that of normal lymphocytes

Total nucleotides and RNA

The mean $F_{tot}265$ as well as the intercellular variability in the $E_{tot}265$ per cell was greater in AL than in CLL or normal lymphocyte populations (table

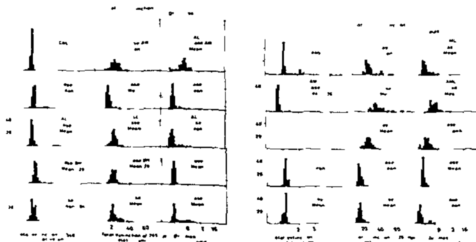


Fig 3 Frequency distribution of DNA $E_{tot}265$ and dry mass per cell in populations derived from individual cases of leukemia (see text)

I, figs 2, 3) The greatest mean $E_{tot}265$ values were observed in cell populations derived from AML, lower values were found in ALL and AUL, and the lowest values were found in CLL

The RNA content of normal lymphocytes (circa 10 % of the total nucleotide content) has been assumed to be negligible (26) The cells in the populations derived from CLL contained similarly negligible amounts of RNA (table II) The mean RNA DNA ratio in all three sub groups of AL was variable, but in most instances was considerably greater than in CLL and normal lymphocytes The lowest RNA DNA ratio within the AL group was found in the cell populations derived from a patient with ALL (M B table II), in which the cells did not appear to contain a measurable amount of RNA, and the largest mean RNA DNA ratios were found in the cell populations derived from two cases of AML (M J and N S, table II)

Mass

The lowest mean mass values were found in cell populations derived from patients with CLL and in normal lymphocytes (table I) The mean mass values were larger in all cell populations derived from patients with AL but there was no clear difference between the mean mass values observed in cell populations derived from the various diagnostic sub-groups There was a marked intercase variability in the mean mass values of individual cell populations derived from patients with AL The intercellular variability within a given population was, with few exceptions, of the same order of magnitude as the intercellular variability of the total nucleotide content In the case of cell populations derived from patients J D and A C, in which there was a relatively larger intercellular variability in mass these variations could at least in part be attributed to contamination of the population with polymorphonuclear leukocytes which had larger mass values (table I)

Discussion

The frequency distributions on the basis of DNA content per cell observed in these studies are in essential agreement with previous observations (23, 24, 40, 41, 49), but cells with a DNA content greater than that of normal diploid cells were encountered much less frequently in the cell populations derived from patients with AL than in populations of either normal or neoplastic cells growing asynchronously in culture (25) In the present studies, cells with an intermediate or 4c-DNA content probably were in the S or G-2 phase of DNA synthesis, or perhaps were aneuploid cells, which are known to occur in cell populations derived from ALL (22, 44) Other studies (2, 12, 18, 28 29) utilizing autoradiographic techniques also have indicated that the percentage of DNA synthesizing leukemic cells is low in AL as compared to the percentage of such cells in populations of blast cells derived from normal bone marrow There also is evidence that the percentage of DNA synthesizing cells in the bone marrow of patients with AL is somewhat higher than in the peripheral blood

buffy coats of such patients (28, 29 35) These observations have been interpreted in various manners for example Craddock and Nakai (12) postulated a prolonged G-1 (in relation to S-1 and G 2) phase for AL cells while Killman (28) suggested the presence of non dividing, "mature" blast cells in the peripheral blood of patients with AL, as evidenced by the more rapid rate of incorporation of H^3 thymidine by large "mature blast cells than by small blast cells (19), and the failure of atypical blast cells to incorporate this DNA precursor It is evident that these explanations are not necessarily mutually exclusive since a cell with an infinitely long G-1 phase may be considered to be a non dividing cell Although the autoradiographic and ultra microspectrophotometric evidence supports this interpretation there is no proof that such non dividing (unlabeled or 2 c) cells will not divide at some genetically determined time, upon appropriate stimulation It is of particular interest that this low frequency of cells in DNA synthesis has no parallel in continuous cultures of human lymphoblasts derived from the peripheral blood buffy coat of a patient with acute lymphoblastic leukemia (16, 37) Nearly all of the cells in these cultures which have been maintained in continuous asynchronous log phase growth for more than eighteen months actively synthesize DNA 36

The results of previous studies (20 21) on the total nucleotide content of AL cells are in general accord with the present observations The observed inter cellular variation of both total nucleotide and protein content was greater than would be expected in homogeneous non-dividing populations of normal lymphocytes and is of the same order of magnitude as that found in continuous asynchronous cultures of human lymphoblasts derived from the peripheral blood buffy coat of a patient with acute lymphoblastic leukemia (36) This discrepancy between the DNA content and the total nucleotide and protein content per cell implies that a portion of the cells in these populations were synthesizing RNA and protein although they were non DNA synthesizing and non-dividing suggesting an abnormal maturation process Such non-dividing RNA and protein-accumulating cells have been described in populations of other kinds of neoplastic cells maintained in culture (25 26) Biochemical studies (30, 31, 46) have suggested a considerably increased RNA and protein synthesizing activity in leukemic cells as judged by the more rapid incorporation of RNA and protein precursors as compared to normal cells despite the low frequency of DNA-synthesizing cells in such populations Studies on the incorporation of labeled uridine and phenylalanine (45) also have indicated the non-uniform synthesis of RNA and protein in non-dividing leukemic cells Indeed in these studies 45 the marked incorporation of labeled uridine by some cells was suggested as biochemical evidence for the presence of a a replicating RNA virus Other possible explanations of the increased intercellular cytochemical variability in populations of human leukemic cells in vitro have been discussed elsewhere in relation to a

similar increased intercellular cytochemical variability observed in lymphoma cells from the thymus and lymph nodes of AKR mice (17) The relevance of any of these explanations, however, to the reasons underlying the increased intercellular cytochemical variability observed in human leukemic cells in vivo is not clear at the present time Although similar cytochemical patterns were found in cell populations derived from some patients, there was considerable intercase variability among the cell populations derived from patients with AL Thus far too few cases have been studied to permit conclusion as to the diagnostic value of these cytochemical parameters for the differentiation of various sub-groups of AL The degree of intercase variability however suggests the possibility of differentiation into sub groups on the basis of cytochemical rather than morphologic criteria

The distribution patterns of total nucleotide and DNA content per cell in cell populations derived from patients with CLL is in agreement with the concept that most of these leukemic cells are mature non DNA-synthesizing, and non proliferating as was concluded previously from autoradiographic studies (12) The small intercellular variability in the total nucleotide content corresponds with the small variability in the DNA content and mass values in the cell populations derived from patient G D In the other case of CLL (A C) a small percentage of the cells were characterized by larger mass values which perhaps may be attributable in part to possible contamination with polymorphonuclear leukocytes (95 % of the cells were lymphoid) However increased mass values have been noted previously (13) in lymphocytes derived from patients with CLL as compared to normal lymphocytes, and in one instance, the distribution pattern of the mass values was similar to that observed herein in the cell populations derived from patient A C As judged by the incorporation of H^3 thymidine (12) a small proportion (0—1 8 %) of the cells from cases of CLL synthesized DNA although there was considerable variation between different cases Differences in the distribution patterns of mass values in the cell populations derived from patients G D and A C probably reflect the presence of a small proportion of growing cells in the former, and a somewhat larger proportion of such cells in the latter populations The patterns of distribution of cytochemical values in cell populations derived from CLL thus clearly differ from those observed in cell populations derived from AL with respect to DNA total nucleotide and protein content per cell, while the cytochemical differences between cell populations derived from CLL and populations of normal lymphocytes were less clear, and perhaps were in some instances limited to differences in mass values per cell

The mean values for DNA total nucleotide, proteins and the RNA DNA ratios determined for the cell populations considered herein are in general agreement with previous biochemical determinations of these parameters (14 38 43) on populations of similar cells The usual RNA DNA ratio in AL cells varies from 0 3—1 0 while the ratio in

CLL cells and normal leukocytes is somewhat lower The greater RNA content of leukemic blast cells as compared to the RNA content of normal mature WBC is consistent with the earlier cytophotometric observations described by Thorell (48) who reported that the RNA content of blast cells, whether normal or leukemic, was greater than the RNA content of mature leukocytes The somewhat larger RNA DNA ratio observed in populations of AML cells as compared to those derived from ALL is of interest, but is of doubtful significance with respect to diagnostic differentiation because of the variation between cell populations derived from individual patients There are too possible explanations other than real quantitative differences in the RNA DNA ratios of AML and ALL cells For example the low mean Feulgen DNA value of the population of AML cells derived from patient N S probably is not an accurate estimate of the DNA content of these cells thus resulting in an exaggerated RNA DNA ratio Presuming the lowest modal chromosome number 42 chromosomes) reported in AML 44 it is unlikely that a 10 % reduction of the normal modal chromosome number would explain the 26 % reduction in the mean DNA content of these cells A more likely explanation might be differences in the rate or extent of acid hydrolysis of DNA in these leukemic cells as compared to normal lymphocytes Such differences between normal and neoplastic cells with respect to the optimal time for the hydrolysis of DNA have been reported 1 3 Differences in optimal time of hydrolysis also might explain the circa 10 % increase in the "basic Feulgen DNA value in leukemic cells Alternative explanations of course are that the modal chromosome number is increased 10 % in these leukemic cells (44) or that the chromosomes of leukemic cells contain circa 10 % more DNA as may be the case in other kinds of neoplastic cells (42)

It is evident from the studies considered herein that cell populations derived directly from patients with AL differ from populations of normal lymphocytes (and from cell populations derived directly from patients with CLL) with respect to the degree of intercellular variability in total nucleotide and protein content per cell It is of interest too, that a similar degree of intercellular variability in these cytochemical parameters was observed in human lymphoblasts maintained in continuous cultures derived from the peripheral blood buffy coat of a patient with acute lymphoblastic leukemia 36 Previous studies on the cytochemical variability of neoplastic cells (6 7 25—27 50 were concerned with cell population in which the individual cells were characterized by a relatively high cytoplasm nucleus ratio The degree of variability in the cytoplasmic RNA and cytoplasmic protein content of such cells could be determined without correction for the relatively small nuclear protein and nuclear RNA content The scanty cytoplasm characteristic of most leukemic cells precludes the possibility of direct determination of the degree of cytoplasmic variability making valid comparisons with the results of earlier

experiments with cytoplasm rich cells difficult In the present studies, attempts were made to determine the total nucleotide and protein content of the nucleus and cytoplasm separately, but such attempts proved to be unfruitful with these kinds of preparations

The results of the present studies, like those with other kinds of neoplastic cells (6, 7), human lymphoblasts in culture (36), and lymphoma cells from the thymus and lymph nodes of AKR mice (17) suggest that there are underlying disturbances or dysfunctions in the cellular mechanisms concerned with nucleic acid and protein synthesis in neoplastic cells In view of the evidence relating the principal nucleolar system to these cellular mechanisms (6 7) and the many evidences of marked nucleolar disturbances in actively invasive tumors (11), the scanty cytoplasm and the relatively large nuclear mass characteristic of leukemic cells makes study of nuclear and nucleolar variability of particular interest Such studies with human lymphoblasts in continuous culture are now in progress

Summary

The DNA, total nucleotide and protein content per cell has been determined in populations of normal and leukemic human leukocytes by means of combined microinterferometry and microspectrophotometry

Populations of cells derived directly from patients with acute leukemia exhibited a greater degree of intercellular variability as well as a greater RNA DNA ratio than did normal human lymphocytes or populations of cells derived directly from patients with chronic lymphocytic leukemia The majority of normal human lymphocytes and the cell populations derived directly from patients with chronic lymphocytic leukemia appear to be non proliferating Cell populations derived directly from patients with acute leukemia contained proliferating cells in which the G 1 phase, in relation to the S and G 2 phases appears to be prolonged as compared to the growth patterns exhibited by human lymphoblasts maintained in continuous culture A considerable proportion of the cells in populations derived directly from the peripheral blood of patients with acute leukemia apparently were non DNA synthesizing and non proliferating, but there was evidence of continued RNA and protein synthesis in such cells, suggesting an abnormal maturation process

There was considerable variation in the cytochemical patterns of cell populations derived directly from individual patients with acute leukemia, hence no conclusion could be drawn relating cytochemical differentiation to different morphologic sub groups of acute leukemia The degree of intercase variability, however, suggested the possibility of differentiation into sub groups on the basis of cytochemical rather than morphologic criteria

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From the Institute for Medical Cell Research Karolinska Institutet Stockholm Sweden

Glycogen Synthesis in Normal, Leukemic and Polycythemic Leukocytes

Preliminary report of a quantitative cytochemical study

By

G GAHRTON and A ZETTERBERG

Biochemical studies have previously shown that mixed myeloid blood cells in chronic myelocytic leukemia (CML) contain on an average lower amounts of glycogen than normal leukocytes (11, 14, 17, 18) Microspectrophotometric determinations of the amounts of α amylase labile periodic acid Schiff reactive material (PASMa) in single cells in populations of CML-leukocytes have later shown that this reduction in glycogen amounts was due not only to the presence of immature glycogen-poor myeloid cells but also to a reduction in the glycogen content in mature CML-neutrophil leukocytes compared to normal neutrophil leukocytes (9, 10) Biochemical work has shown that the average rate of glucose incorporation into glycogen of CML-leukocytes was higher than into glycogen of normal leukocytes in vitro (11 14 16) The purpose of the present work was firstly to study whether this augmented rate of glycogen synthesis was assigned to a certain population of CML leukocytes, and secondly to study whether there was any relation between the amounts of glycogen in single cells and the rate of glycogen synthesis in the same cells Such studies were made possible by using combined microspectrophotometric and autoradiographic techniques

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Material and methods

The clinical material was obtained from the Medical Clinic University Hospital Lund Sweden and the Radiumhemmet Karolinska Sjukhuset Stockholm Sweden (Drs A Nordén and S Franzen)

Two cases of CML and two cases of polycythemia vera were investigated Leukocytes from the same normal subject were used as references in all experiments White blood cells from venous blood were roughly separated from red blood cells with 1 per cent fibrinogen according to a previously described method (4) The leukocyte rich supernatant was centrifuged at 300 g for 7 min and the cells resuspended in the patients own serum to a leukocyte count of about 15 000 cells per mm³ One ml of the

TABLE I. Total extinction at 546 mμ (E_{tot}NL) of individual PAS stained neutrophil leukocytes and grain counts in the same cells after incorporation with glucose-6-³H (see text)

Experiment	Case	Incubation time (min)	E_{tot}NL Mean	E_{tot}NL Coefficient of variation	Grain count Mean	Grain count Coefficient of variation
1	CML M J	10	63 ± 1.7[1]	19	15.4 ± 2.0[1]	93
	Normal	10	99 ± 2.3	17	6.2 ± 0.3	32
2	CML M J	40	62 ± 2.5	28	27.7 ± 3.4	87
	Normal	40	95 ± 2.3	17	7.0 ± 0.3	34
3	CML B N	10	82 ± 2.8	24	8.5 ± 1.0	83
	Normal	10	107 ± 3.1	20	5.1 ± 0.4	56
4	Polycythemia vera F W	10	93 ± 4.4	33	4.6 ± 0.3	44
	Normal	10	94 ± 3.2	24	4.0 ± 0.2	38
5	Polycythemia vera K B	10	121 ± 4.3	23	4.2 ± 0.4	61
	Normal	10	102 ± 2.9	20	5.5 ± 0.3	33

[1] Standard error of the mean. (Number of cells = 50.)

cell suspension was incubated at 37 C with 0.1 μC glucose-6-³H (specific activity 1.45 mC/mg) in 0.1 ml saline for 10 min or 40 min. The cells were then washed twice in saline, centrifuged at 300 g for 7 min and resuspended in their own serum for preparation of smears. They were fixed in absolute methanol for 60 min, stained by a modified McManus periodic acid Schiff (PAS) reaction (6, 8) and measured in the rapid scanning microspectrophotometer (1, 2, 3, 12) at 546 mμ to obtain the total extinction (E_{tot}) of single cells. Only mature neutrophil leukocytes were measured since precursor cells usually contained too low concentrations of PASMa to be accurately identified and measured. E_{tot} of single neutrophils (E_{tot}NL) was a measure of the amounts of PASMa, predominantly glycogen in these cells (7, 8). Autoradiographs were thereafter prepared by the stripping film technique (5, 15) and grain counts made. The number of grains per unit area of the cell was used as a measure of the amount of glucose incorporated per mass unit in the fixed cells since the thickness of the cells was more than 0.05 mg/cm² as measured by microinterferometry (13). Determinations of the amount of PASMa and grain counts were in all cases performed in the same 50 individual cells. Control specimens digested with α-amylase were investigated in all subjects.

Results and discussion

The grains of neutrophil leukocytes were to about 80 per cent located in the α-amylase labile and PAS positive part of the cytoplasm in all cases. PAS negative red blood cells and lymphocytes did not contain significant numbers of grains. Myeloid precursor cells in CML showing a weak PAS reaction incorporated glucose 6—15 times (depending on the degree of immaturity) slower than mature CML neutrophils. Glycogen synthesis was thus related to the PAS positive parts of the leukocytes, though there was no correlation between the

amounts of PASMa per neutrophil leukocyte and the number of grains in the same individual cells either in normal leukemic or polycythemic cases The rate of glycogen synthesis therefore did not seem to be related to the amounts of glycogen per neutrophil leukocyte

The mean incorporation rate into CML neutrophil leukocytes was 1 6—4 0 times higher than into normal neutrophils and the intercellular variation in incorporation rate in CML-neutrophils was much larger than in normal neutrophils (table I) These results indicate that the rate of glycogen synthesis was on an average considerably higher, though much more variable among the individual neutrophils in the CML population than in the normal population, despite the fact that CML-neutrophils contained lower glycogen amounts with an approximately normal intercellular variability

Neutrophils from patients with polycythemia vera did not show clear differences from normal with respect to the rate of glycogen synthesis (table I) A somewhat augmented average rate of glycogen synthesis has previously been demonstrated by biochemical methods (11 14) The size of the present material, does not however, permit definite conclusions about differences of the order of magnitude demonstrated in these studies

To sum up the results show that there are differences in the glycogen metabolism between mature CML-neutrophils and normal neutrophils Further studies of the mechanisms responsible for these differences are in progress

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From the Medical Department B (Head A Tybjærg Hansen, M D) Rigshospitalet the University of Copenhagen Copenhagen Denmark

Cardiac Arrest

Integrated Treatment with Drugs and Countershock or Pacemaker

By

P Fritz Hansen and Erik Sandoe

Cardiac arrest is best defined as a sudden *failure of the pumping function of the* heart The cause may be ventricular fibrillation, asystole extremely rapid or extremely slow heart action On more rare occasions cardiac arrest may occur in consequence of a so called acute cardiac collapse , i e a sudden decrease in the contractability of the heart in spite of the fact that the impulse system and the conducting musculature are functioning normally The abolition of the pumping activity of the heart leads to a cessation of arterial pulsation and, in the course of 5—15 seconds to loss of consciousness Hence, sudden loss of consciousness and the disappearance of the arterial pulsation are necessary and satisfactory criteria for the diagnosis of cardiac arrest When cardiac arrest occurs, the patient turns pale or cyanotic and brief episodes of convulsions may start The cardiac arrest may be preceded by respiratory arrest or the respiration ceases within approximately one minute

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Until a few years ago it was outside the range of possibility to intervene actively in this situation To day simple methods have been developed for establishing artificial circulation and ventilation and methods of treatment have been introduced using electrical devices and drugs by means of which the normal pumping function of the heart can be restored

Establishment of artificial circulation and ventilation

Two simple methods are available for the establishment of artificial circulation viz external and internal cardiac massage During external cardiac massage the chest is compressed rhythmically and the heart is by this means squeezed between the sternum and the spine For the purpose of internal cardiac massage a thoracotomy has to be done the pericardial sac is opened and direct massage of the heart is instituted Artificial ventilation may be performed by the mouth to-nose method or by using a Ruben respirator (a rubber mask which

TABLE I Resuscitation in 47 cases of cardiac arrest which occurred in medical department B of the University Hospital Copenhagen Artificial circulation was established by means of external cardiac massage

Cause	No of patients		
	Treated	Resuscitated initially	Discharged
Myocardial infarction	28	16	9
Coronary sclerosis	6	3	1
Pulmonary embolus	3	0	0
Diagnostic studies	4	3	3
Quinidine treatment	1	1	1
Other causes	5	2	2
Total	47	25	16

is connected to a non rebreathing valve and a self-expanding rubber bag) With regard to the details of the technique of cardiac massage and artificial ventilation the reader is referred to the very comprehensive papers published on these subjects 20 21 29 46)

By combining external cardiac massage and artificial ventilation it will in most cases be possible to obtain normal or almost normal arterial oxygen saturation and the systolic blood pressure can be maintained at about 100—110 mm Hg 33) The average cardiac output was 0 6 l per sq metre per minute whereas considerably higher values were recorded during internal cardiac massage 10) Practical clinical experience shows that the cardiac output produced by external massage is sufficiently high to avoid irreversible ischaemic damage to brain heart kidneys and liver This applies also in cases where it is necessary to continue external cardiac massage for longer periods of time For instance we have seen complete restitution in two patients who were treated for ventricular fibrillation by external cardiac massage for periods of 45 minutes and 3 1/2 hours, respectively Furthermore external cardiac massage has the advantage over the internal method in that it is less traumatizing and can be started immediately without any loss of precious time Consequently, we find that the external cardiac massage should be preferred to the internal method even if cardiac arrest occurs in the operating theatre provided that the chest has not already been opened

The various techniques available for the restoration of spontaneous cardiac function will be described later

Results of resuscitation up to the present time

During the years 1964—1966 resuscitation was attempted in 47 cases of cardiac arrest in the Medical Department B of the University Hospital, Copenhagen The resuscitation was performed in the emergency room or in the wards External cardiac massage and artificial ventilation by means of a Ruben respirator were applied Twenty five of the 47 patients were resuscitated initially, 9 died later and 16 were discharged after successful resuscitation I e the net result of the resuscitations was 34 % (table I) In 28 cases the cardiac arrest was caused by an acute myocardial infarction Sixteen of these

TABLE II Resuscitation in cases of cardiac arrest occurring in wards or emergency rooms Artificial circulation was established by means of external cardiac massage

Authors	No of patients Treated	Discharged	Percentage
Bjork & al (4)	69	12	19
Johansson (19)	65	13	20
Jude & al (20)	224	35	16
Sykes (48)	68	15	22
Balslov & al (2)	49	9	18
Pedersen & al (34)	123	7	6
Smith & al (42)	126	21	17
Stemmler (45)	103	5	5
Own material 1966	47	16	34
Total	874	133	15

TABLE III Cardiac arrest caused by myocardial infarction

Authors	No of patients Treated	Discharged	Percentage
Biorck & al (4)	45	2	4
Johansson & al (19)	26	2	8
Jude & al (20)	61	9	15
Balslov & al (2)	25	3	12
Pedersen & al (34)	40	0	0
Robinson & al (36)	53	11	21
Semple & al (40)	20	8	40
Smith & al (42)	50	8	16
Stemmler (45)	26	1	4
Own material	28	9	34
Total	374	53	14

patients were resuscitated initially, 7 died later and 9 were able to resume their normal activities In 3 patients with pulmonary embolism it was impossible to produce any palpable pulse by cardiac massage Three cases of cardiac arrest occurred in connexion with diag nostic studies and in two of these cases resuscitation was successful The third of these patients was a young girl with cyanotic heart disease and severe pulmonary hypertension Two hours after right cardiac catheterization she de veloped ventricular fibrillation and all attempts at restoring circulation were unsuccessful

Data obtained in series of patients comparable to our material are summa

rized in table II One hundred and thirty three (15 %) patients out of a total of 874 patients with cardiac arrest were discharged Of 374 patients with myocardial infarction and cardiac arrest, 53 were discharged (14 %) However, the results of the different series show wide variations (table III) Cardiac arrest in case of pulmonary embolism appeared to be particularly difficult to handle (45) As previously mentioned, our series included 3 patients with pulmonary embolism, and in all 3 cases it was impossible to restore arterial pulsation by means of external cardiac massage One successful episode of resuscitation in case of cardiac arrest during pulmonary embolism has been published by Hasager Boss and Larsen (18)

The duration of time from the occurrence of the cardiac arrest and to the start of resuscitation measures influenced the results greatly (34) This may explain to a certain extent why cardiac arrest occurring in operating theatres or in recovery rooms, where the patients are constantly supervised by medical personnel, carried a much better prognosis than cardiac arrest occurring in the wards (20) Cardiac arrest lasting for more than 4—6 minutes will usually lead to irreversible brain damage Therefore, it might be feared that the wide clinical application of the technique of resuscitation will result in the survival of an increasing number of decerebrated individuals However, this has not been the case Decerebration after resuscitation has been observed, but the majority of these patients died within a few days or weeks In the present material only one patient with slight cerebral reduction was observed This patient was a 70 year old man with arteriosclerotic heart disease, who suddenly developed ventricular fibrillation during his stay in the ward, and in whom resuscitation was not started till about three minutes had passed

In which cases should resuscitation be attempted?

Of course it is not justifiable from a medical or moral point of view to try to restore circulation in all persons who die The indication for treatment must be that the cardiac arrest occurs suddenly and unexpectedly (20) According to this formulation there is no indication for resuscitation in patients who are extremely ill from chronic diseases, or in patients in the terminal stages of a disease

The time factor must be taken into account If circulation has been stopped for 4—6 minutes, it will be impossible, as previously stated, to restore cerebral function If the exact time of the cardiac arrest is unknown, it is more difficult to evaluate the situation, and actually there exist no criteria on which to decide whether the cardiac arrest has lasted e g 2 or 8 minutes In such cases there is only one thing to do to start resuscitation

When can an attempt of resuscitation be stopped?

This is a very difficult question Factors as the patient s underlying disease the condition prior to the cardiac arrest, and the period of time passing before resuscitation was started must be taken

into consideration If the external massage does not produce any palpable pulse treatment with internal cardiac massage should be considered If the pupils remain dilated in spite of effective cardiac massage, the attempt can soon be abandoned in most cases If, on the other hand, the pupils contract or remain contracted there is every reason for continuing resuscitation even for a very long time

If the pupils become dilated during the cardiac massage, it will usually be a sign of irreversible brain damage Also in this respect however surprising events may occur Thus, in one of our patients resuscitation was abandoned after the pupils had been maximally dilated for 20 minutes At this time the ECG-complexes were satisfactory, the pulse was extremely weak and blood pressure was unrecordable The following morning the patient was awake and cerebral function was intact In this patient the dilation of the pupils was presumably a result of the use of large doses of aramine (cf 12)

Finally, it should be mentioned that the prognosis need not be poor, even if the cardiac arrest is followed by prolonged unconsciousness One of our patients was unconscious for 3 days *after the cardiac arrest*, but was later completely restored Other authors have made similar observations (35, 45)

Procedure in the treatment of cardiac arrest

In this section the procedures are outlined which are followed at present in the treatment of cardiac arrest in the Medical Department B the University Hospital Copenhagen

I *The diagnosis of cardiac arrest is verified*

I e it is examined whether the pulsation in the arteries of the neck has stopped

II *The exact time of the occurrence of the cardiac arrest is noticed*

III *The patient is placed on a rigid surface and free airways are secured*

IV *External cardiac massage and artificial ventilation are started*

Cardiac massage is applied at the rate of 60—90/minute alternately 2—3 ventilations and 15 chest compressions are given The ventilation is initiated by the *mouth to-nose method* and as soon as possible we change over to the Ruben respirator and pure oxygen *is given As soon as the anaesthetist* arrives the patient is *intubated* When the artificial ventilation and the cardiac massage are established the supply of oxygen to the tissues is restored and consequently there will be relatively ample time for the subsequent application of electric treatment and drugs It should be emphasized however that during this treatment the *artificial ventilation and cardiac massage must not be stopped for more than 5—6 seconds*

V *Adrenaline 1 mg in adults (in children 0 02 mg/kg)*

The drug is given by intravenous injection or if this is impossible by intracardiac injection

VI *Intravenous drip infusion of sodium bicarbonate*

We use an 8 4 % solution of sodium bicarbonate (1 ml of the solution containing 1 mEq of bicarbonate) 100 ml of the solution are given immediately For each 10 minutes duration of cardiac arrest another 50—100 ml are infused (in children 1 ml/kg/10 min) The treatment with bicarbonate counteracts the acidosis which inhibits the contractability of the myocardium and provokes abnormal impulses in the heart (22 23)

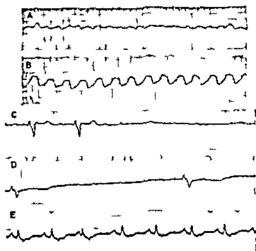


Fig 1 Electrocardiograms during cardiac arrest A Ventricular fibrillation (46-year-old male with septal infarction) B Ventricular tachycardia (52 year-old male with posterior wall infarction) C Asystole (44 year old *male with arteriosclerotic heart disease who* suddenly developed complete atrioventricular block) D Bradycardia (67 year old male with arteriosclerotic heart disease) E Cardiac collapse (45 year-old female with anterior wall infarction Cardiac arrest because of ventricular fibrillation Sinus rhythm was reestablished by means of countershock but in spite of the sinus rhythm the patient is still pulseless Thoracotomy was performed with a view to internal cardiac massage and it was observed that the entire anterior wall of the left ventricle was paralysed because of the infarction whereas the posterior wall showed contractions synchronously with the relatively normal complexes seen in the ECG)

VII *The cause of the cardiac arrest is clarified by ECG*

The ECG may show either ventricular fibrillation ventricular tachycardia asystole bradycardia or it may show relatively normal conditions (fig 1) The last tracing means that the cardiac arrest is the result either of an acute cardiac collapse acute hypovolaemia or vasodilatation In acute cardiac collapse the myocardium is too weak to produce an arterial pulse, in spite of normal stimulation In acute hypovolaemia or vasodilatation the venous return to the heart is too small

VIII *Restoration of spontaneous cardiac function*

1 *Treatment of ventricular fibrillation*

A D C countershock from 100 to 400 wattsec (in children 50—200 wattsec) or if only an alternating current type defibrillator is available, A C countershock from 440 volts up to 640 volts are given (in children 220 volts) If some time has passed since the adrenaline injection was given the injection should be repeated before the countershock is applied since animal experiments have shown that it is much easier to defibrillate if adrenaline has been administered immediately before (31) If the heart continues to fibrillate, a 'pre treatment with an antiarrhythmic drug should be given, followed by a new attempt at defibrillation with countershock The drugs which we have found particularly *useful as pre treatment to defibrillation with* countershock are listed in sections B-E

B *Antazoline* (Antistina®) 300—400 mg and — if required — *adrenaline* 1 0 mg intravenously (in children antazoline 5 mg/kg and — if required — adrenaline 0 02 mg/kg)

Antazoline is known to be a potent antiarrhythmic drug (1 11) In our material this agent has in several cases made the heart more susceptible to electric defibrillation *when other agents have failed* At the moment we regard antazoline as the drug of choice in ventricular fibrillation

C *Lidocaine* (Leostesin®) 100—150 mg and *adrenaline* 1 0 mg intravenously (in children lidocaine 2 0 mg/kg and adrenaline 0 02 mg/kg) (7, 17)

D In case of ventricular fibrillation resulting from *hyperpotassaemia* the most effective treatment would be intravenous infusion of *10(—20) ml of a 10 % solution o calcium chloride* (47) However in case o digitalis intoxication and hypopotassaemia calcium chloride is contraindicated (15 47)

E *Propranolol* (Inderal®) 2—5 mg (up to 10 mg if required) given slowly by the

intravenous route Propranolol should be particularly effective in digitalis intoxica tion but might be used also in cases of ven tricular fibrillation of other aetiology (14 41) Overdosage results in asystole or acute myocardial failure and we are somewhat reluctant to use it after having seen the development of irreversible asystole in 2 patients following 20 mg of propranolol given by mouth The antidote to overdosage of propranolol is isoprenaline

F *Potassium chloride* 50 mEq in 1 000 ml of isotonic glucose solution is infused intra venously in the course of 2—3 hours (43) Several authors recommend 40—60 mEq of potassium chloride in 1 000 ml of a 10—16 % glucose solution to which is added 20—40 I U of insulin in order to expedite the cellular uptake of potassium (13 30 44) The principal indications for treatment with potassium are hypotassaemia and digitalis intoxication (43)

These injections of anti arrhythmic agents may be repeated but as several of the sub stances possess an additive effect they must not be given in too rapid succession It will often be necessary to continue the cardiac massage for some time after spontaneous cardiac activity has been restored

It must be pointed out that countershock is the most effective therapy in ventricular fibrillation and that it should be given as quickly as possible Thus if a defibrillator is available when the cardiac arrest occurs the effect of one single countershock should be tried before cardiac massage is instituted

2 *Treatment of ventricular tachycardia*

The procedure is the same as in the treat ment of ventricular fibrillation If the D C defibrillator is fitted with a synchronizer the countershock should be synchronized on the R wave of the ECG whereby the risk of provoking ventricular fibrillation is diminished (32)

3 *Treatment of asystole and bradycardia*

A *Isoprenaline sulphate* 0 06—0 2 mg intra venously (36) or *adrenaline* 1(—2) mg intra venously in adults (we have no practical experience concerning children s dose of isoprenaline initial dose of adrenaline in children is 0 02 mg/kg)

B *Intravenous drip infusion of isoprenaline sulphate* (5 mg isoprenaline sulphate in 1,000 ml of isotonic glucose) or if necessary, *intravenous drip infusion of adrenaline* (4 mg of adrenaline in 1 000 ml of isotonic glucose) (51) The drip rate is determined according to the effect 20 drops per minute will be suitable in most cases Overdosage results in tachycardia and possibly in ventricular fibrillation

C *Electric pacing* In many cases it will be necessary to combine the drug treatment with electric pacing First *external pacing* is attempt ed through the intact chest wall It is quite easy to carry out external pacing but accord ing to our experience it is often ineffective and then internal pacing must be attempted The *internal pacing* can be performed by means of an electrode catheter which is introduced into the right atrium and from there into the right ventricle from a peripheral vein (6 16 39) The procedure is not accompanied by any greater risk and offers optimum possi bilities for pacing the heart but the introduc tion of the catheter is time-consuming and requires special training As a last resort one of the ventricles of the heart can be punctured with a wide bore needle Through the needle a teflon covered steel stylet is introduced into the myocardium e g Elecath pacing stylet from the Electro-Catheter Corp and by means of this internal pacing of the heart can be carried out (24 37) In one single case we used this procedure (cf fig 2)

4 *Treatment of acute cardiac collapse*

It is extremely difficult to cope with this condition However it must be emphasized that effective cardiac massage and artificial ventilation continued for a period of time will often — by itself — result in a better myocardial function The following drugs may be tried

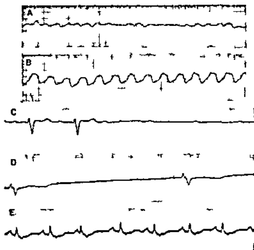


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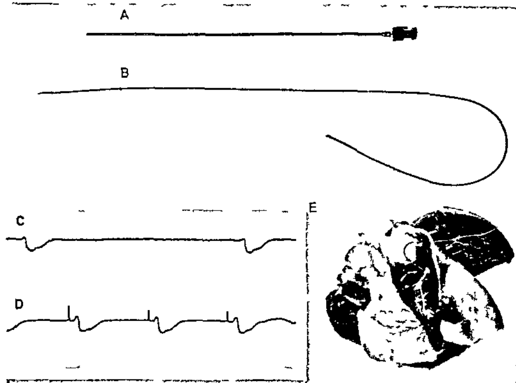


Fig 2 Electric pacing through percutaneously introduced electrode The condition was complicated by a cardiac collapse and attempts at restoring stable circulation were unsuccessful A The needle used for the puncture of the heart B The electrode C ECG immediately before electric pacing showing (extreme) bradycardia D ECG during electric pacing E The heart at autopsy The electrode passes through the myocardium in the infundibulum of the right ventricle and its tip enters the ventricular septum

A *Isoprenaline sulphate* 0 2 mg or *adrenaline* 1—2 mg intravenously or intracardially (we have no practical experience as to children s dose of isoprenaline the initial dose of adrenaline in children is 0 02 mg/kg)

B Intravenous drip infusion of *isoprenaline* (isoprenaline sulphate 5 mg in 1 000 ml of isotonic glucose solution) or of *metaradrine* (Aramine®) (metaradrine 100—200 mg in 1,000 ml of isotonic glucose) or of a *mixture of isoprenaline and metaradrine* Isoprenaline results in a pronounced stimulation of the myocardium, the heart rate is increased and moderate peripheral vasodilatation occurs whereas metaradrine produces a more moderate stimulation of the heart and pronounced peripheral vasoconstriction After having started treatment with isoprenaline it may, therefore be necessary to combine with metaradrine and sometimes to change to infusion of metaradrine only because of vasodilatation or tachycardia In cases of primary vasodilatation metaradrine is used alone This pressoramine treatment is still much discussed (3 5 8 25 27 38 49) It is known however that both isoprenaline metaradrine and other pressoramines increase the oxygen consumption of the myocardium and these agents should therefore always be given in as small quantities as possible This means that a blood pressure at the lower normal limit (80—90 mm Hg systolic) must be considered to be satisfactory and the intravenous drip infusion should be stopped if the blood pressure increases further

C If the patient has not already been given a digitalis glycoside he should be given 0 5 mg of ouabain intravenously (in children 0 04 mg digoxin intravenously)

After successful resuscitation the patient must be supervised very carefully Blood samples for acid base studies electrolyte determinations and blood urea measurement should be taken The hourly urinary output should be followed with a view to an early diagnosis of shock kidney The patient should be examined with a view to possible complications to the cardiac massage (rib fractures pneumothorax, intra abdominal haemorrhage) Among our patients one had liver damage several patients had rib fractures and one had a sternal fracture In one case the chest had to be fixed because of respiratory failure If the cardiac arrhyth mia shows a tendency to recur prophylac tic antiarrhythmic treatment should be con sidered Drugs as quinidine and propranolol can be used or if necessary infusion of a potassium chloride solution to which are added insulin and glucose (13, 30 44)

The value of intensive care

Since the time factor is of decisive im portance for the result of the resuscita tion, intensive care units have been established in several medical centres for patients in special risk groups e g myocardial infarction Most of these centres have reported successful results of the intensive care (9 28 50) from one centre the results are more disap pointing (26) Therefore the therapeu tic value of these units is still the subject of discussion However one thing is certain these units will provide us with more exhaustive information about the pathophysiological conditions and the causes of death in acute myocardial infarction and in all probability this will eventually result in new therapeutic advances Furthermore, these units will provide an ideal background for the testing of the various prophylactic methods of treatment in case of cardiac arrest, since the continued recording of the ECG offers the possibility of a complete evaluation of the effect on the heart action of the drugs employed

Summary

The results of treatment in 47 cases of cardiac arrest, occurring in a medical ward are described External cardiac massage was used in the resuscitation Sixteen patients (34 %) could subse quently be discharged without any essen tial sequelae Of 28 patients with cardiac arrest associated with acute myocardial infarction 9 could be discharged

The treatment of cardiac arrest is discussed, and the value of combining electric measures and drugs in the treat ment of the underlying arrhythmias is emphasized In ventricular fibrillation the authors experienced good results with antazoline in some cases where other therapeutic measures failed As regards propranolol the authors observed the development of asystole in two cases and they warn against uncritical application of this agent

On the background of their own experience the authors have made an attempt to lay down lines to follow when deciding upon the time at which resuscitation should be continued and the time at which it can be abandoned It is pointed out that during the resuscita tion procedure the evaluation of the immediate prognosis is difficult

The material as a whole — and in particular the two patients who were completely restored after uninterrupted external cardiac massage for 45 minutes and 3 1/2 hours, respectively — shows that it is possible by external cardiac massage and artificial ventilation to prevent the development of irreversible ischaemic damage to vital structures

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The treatment level of polynuclear white cells was below 2 000/mm³ in two patients and the reduction exceeded 15 % in 12 of the 18 cases The change was probably significant (0 05 > P > 0 02)

The treatment level of total white cells was below 4,000/mm³ in 4 cases and the reduction exceeded 15 % in 11 of 18 cases The changes was probably significant (0 05 > P > 0 02)

Discussion

In this trial hematological findings were unexpected In contrast to several studies on platelet stickiness and other coagulation mechanisms during Atromid treatment comparatively few reports include observations on conventional hematological data In trials where white cell counts and differential counts were followed no significant changes were observed (5, 8 9 21, 23) As mentioned above one case of agranulocytosis — without direct suspicion of causal connection with Atromid — has been reported (20)

Most of the patients during the trial continued treatment with different drugs for cardiovascular disease with consequent risk of influence of such therapy on blood cell counts All determinations were done as routine tests by different examiners Consequently it was not feasible to get long lasting determinations on errors of methods Nor was it suitable to determine normal values for white cell counts in hospital patients where it is difficult to exclude hematological changes from diseases and drugs A study is being carried out on a health control sample on the possible relationship between lipid disorders and white blood counts (7)

The results of the present study suggest a spontaneous tendency to leukopenia in hypercholesterolemia and hypertriglyceridemia Before treatment there was no correlation in this small group of patients between total polynuclear or mononuclear white cell counts on the one side and cholesterol or triglycerides on the other (P > 0 10)

During treatment the reductions in total and polynuclear white cell counts were probably significant This reduction need not be considered as a sign of toxicity of Atromid S to the bone marrow but cold as well be secondary to changes in lipid metabolism

No significant correlation between the reduction of serum cholesterol or triglycerides and the different white cell counts could be found

Apart from the changes in the peripheral blood additional findings give support to the suspicion of hematological changes related to these lipid disorders An increased fat content of the bone marrow may reasonably interfere with hematopoesis Such infiltration was found in the cases 7 and 12 at post mortem examinations In case 16 sternal puncture before Atromid S treatment had shown fatty marrow to an extent of about 40 per cent with an increase of reticulum The spleen was small on X ray examination At post mortem examination in case 7 there was found reduction of the lymphoid apparatus of the spleen and swelling of the reticulum

The signs of disturbance of lymphoid tissue in the last two patients agree

with the impression of low mononuclear white cell counts before treatment in 6 out of 19 cases These 6 subjects all had values at or below 1,700/mm³ According to Wintrobe the average value in adults is 2,475/mm³ and the minimum 1,785 (24)

Other hematological findings were the pathological extension of red bone marrow in case 3 and hyperplastic erythro myelopoesis in the sternal marrow of case 9 after one year of treatment Five months after discontinuation of treatment there was thromocytopenic purpura in case 1, presumably due to thiazide therapy

Liver function tests, including repeated bromsulphalein tests, did not suggest any hepatotoxic effects

A spontaneous tendency to leukopenia, especially low mononuclear white cell counts, was observed in several patients During treatment probably significant reductions of polynuclear and total white cell counts were noticed These changes were not interpreted as a sign of toxicity in the bone marrow but rather as a phenomenon secondary to the change in lipid metabolism during Atromid S treatment

In some patients sternal marrow findings before and during treatment as well as post mortem examinations showed signs of hematological disturbances, such as fatty infiltration of the bone marrow and extension of red marrow to the femoral diaphysis In two patients signs of hypoplastic lymphoid apparatus was present supporting the observation of low mononuclear white cell counts before Atromid S treatment

Summary

Nineteen patients with hypercholesterolemia and in most cases hypertriglyceridemia, were treated with Atromid S during an average time of 13 months

Following the observation of a spontaneous tendency to leukopenia, mean dosage was kept comparatively low, 1 5 g daily

Seventeen out of 19 patients responded with a reduction of serum triglycerides exceeding 10 per cent of the initial value, an average of 38 per cent

Twelve out of 19 patients responded with a reduction of serum cholesterol exceeding 10 per cent, an average of 19 per cent

Duration of treatment was shorter than planned in 7 of the subjects Three patients died from myocardial infarction, leukopenia was the reason for discontinuation in one and lack of co operation the reason in the remaining three patients

Acknowledgement

The Atromid preparations were provided by ICI Ltd (Scanmeda Gothenburg)

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From the Departments of Clinical Chemistry and Clinical Physiology of the University Hospital Lund Sweden

Heparin-induced Diamine Oxidase Increase in Human Blood Plasma

By

R HANSSON, C G HOLMBERG G TIBBLING N TRYDING, H WESTLING and H WETTERQVIST

The ^{14}C putrescine method of Okuyama and Kobayashi (5) for the estimation of plasma diamine oxidase (DAO) has after some modifications been made sensitive enough to estimate the enzyme activity in as little as fifty microlitres of blood plasma (7) Using this method a survey of the DAO variations in hospitalized patients was started During this screening it was observed that patients showed elevated levels of DAO in plasma during or immediately after haemodialysis in vivo This finding was eventually traced to the use of intravenous heparin during haemodialysis (7) The present report contains a closer description of the heparin effect in healthy non pregnant subjects

The intravenous injection of heparin is promptly followed by a rise of the plasma DAO activity As shown in fig 1 a 10 thousand unit dose of heparin (Vitrum) will induce a significant elevation of this plasma enzyme (within 30 seconds) reaching a maximum at about 60 minutes At this time the plasma DAO level is of the same magnitude as during the third trimester of pregnancy In most of the studied cases the plasma enzyme curve appeared biphasic, as in fig 1 and in almost all of them it had a virtually exponential return to normal values within 6 to 12 hours The rapid initial rise did however in some individuals merge into the maximum plateau level without the initial shelf

If tested on the same subject the plasma DAO level was related to the dose of intravenous heparin but variation between individuals was equally clear (figs 2 and 3) This variation did not seem to depend on body weight in our volunteers

Fig 3 shows the DAO response to heparin administered by different parenteral routes The responses are radically different

Since the discovery by P F Hahn in 1943 (4) that heparin injections are

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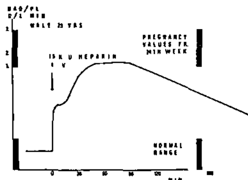


Fig 1 Diamine oxidase (DAO) activity in human blood plasma after intravenous injection of 15 000 units of heparin (Vitrum) given at time zero (indicated by arrows) Black solids refer to physiological ranges of DAO in non pregnant and pregnant adults

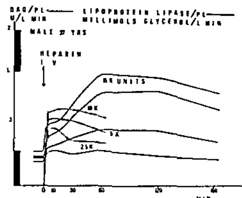


Fig 2 DAO and lipoprotein lipase activity in plasma before and after different intravenous doses of heparin to one subject Solid lines refer to DAO striped ones to lipoprotein lipase Figures refer to kilo-units (K—U) of heparin by intravenous injection at time zero Solids indicate normal values as before

followed by a rapid abolition of alimentary lipaemia a vast literature has accumulated concerning this phenomenon, especially its relationship to the lipoprotein lipase activity in plasma It was therefore of interest to determine the activity of this enzyme in a few of our experiments As seen from fig 2 a dose correlation exists for both, but the lipoprotein lipase and the DAO curves do not run in a parallel fashion

As previously reported (7) heparin has no effect upon the DAO activity of plasma or whole blood in vitro When blood samples, withdrawn from the test subject immediately before the hep-

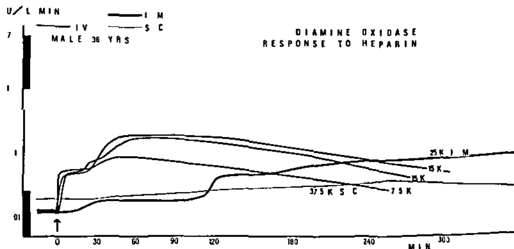


Fig 3 DAO response to heparin injected by different routes S—C = subcutaneous injection striped curve I—M = intramuscular injection interrupted black curve intravenous injection solid curve

arin injection were incubated at 37° C for 5 minutes with blood samples from the post injection period of rapid DAO-increase no effect upon the DAO activity was noticed other than a mere dilution of the enzyme We have shown that the DAO level after heparin also represents histaminase activity Thus we have established a close correlation between the [14]C-putrescine method and determinations of histaminase according to the biological technique described by Ahlmark (8) as well as a chemical method in which the histamine content of the system was measured spectrofluorometrically (6) before and after incubation

Thus it seems reasonable to conclude that there is a large and very rapid DAO increase in blood plasma after intravenous heparin and that this response occurs only *in vivo* The mechanism by which this increase of DAO activity occurs is unknown

That the lymphatic system is a potential transporter of large amounts of DAO activity to the blood stream could be demonstrated in a 37 year old woman, in whom a cannula was inserted into the thoracic duct near the venous angle This was performed in connexion with a lymph gland biopsy following a left sided pulmonary tumour which was shown at later surgery to be a benign bronchioma Small specimens of lymph were obtained with a catheter lag of less than 2 minutes The initial value of DAO activity in lymph varied between 0 5 and 0 9 U per litre per minute

Our normal values for this enzyme activity in human blood plasma 0 005—0 025 U per litre per minute during

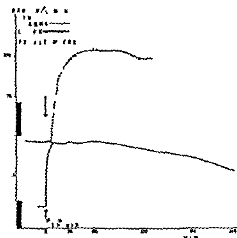


Fig 4 DAO activity in plasma (solid line) and thoracic duct lymph (broken line) before and after intravenous injection of 10 000 units of heparin

pregnancy increasing to final values between 1 and 7 U per litre per minute) This lymph/plasma ratio agrees well with that found in cat by Carlsten et al (2) When 10 thousand units of heparin were given intravenously to the patient (fig 4), the DAO level in the thoracic lymph rose to 140 units per litre per minute This enormous increase followed a curve similar to that in plasma although there was a delay of several minutes and the level was more than a hundred times higher The initial phase of the DAO curve cannot be due to an outflow of enzyme via the thoracic duct but the findings are suggestive of a partial contribution by the lymph to the later DAO elevation in the plasma

The early phase of the DAO increase after heparin might thus be caused by enzyme liberation directly into the blood stream By simultaneous aspiration through multiple catheters in the main

blood vessels, several blood samples for DAO estimation have been obtained during the first minutes after rapid intravenous injection of heparin Our findings at present suggest that the thoracic duct does not mediate the DAO rise in the first phase nor do any other organs that give their blood to the superior vena cava The lungs do not seem to contribute materially to the early activity of this enzyme in plasma The intestinal mucosa, the liver and the kidneys have been shown to be relatively DAO rich organs in man (9) Hitherto, our observations conform with the assumption that the main source of the initial DAO increase lies in these organs

It has been reported (1, 3) that heparin injection into guinea pigs is followed by an increase of histaminase in the blood plasma and a diminished tendency to shock after histamine Evidence was obtained that the histaminase came from the liver These findings are partly analogous to our results in man

Summary

The effect of parenteral doses of heparin on the plasma diamine oxidase (histaminase') activity was studied in healthy adults A large and rapid increase of the enzyme activity occurred after intravenous heparin (5 to 15 thousand units) This effect is similar to that previously found for lipoprotein lipase, but the plasma concentration followed a different time course in our subjects Observations on thoracic duct lymph indicate that it is an important transporter of the diamine oxidase to the blood plasma

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From Oslo University Hospital Medical Department B (Head Ole Storstein M D) Rikshospitalet Oslo Norway

Quinidine Concentration in Serum

The Stability During Maintained Treatment with Two Different Types of Delayed Absorption Tablets

By

Else Marie Lindseth Ditlefsen and Christopher Bjerkelund

The use of DC countershock in the treatment of cardiac arrhythmias has renewed the interest in quinidine A high percentage of patients suffering from atrial flutter and atrial fibrillation can now be converted to sinus rhythm, and this has greatly increased the need for maintained quinidine therapy The risk during prophylactic quinidine thera py is considered small (4 15), compared with that observed during conversion of chronic atrial fibrillation when higher doses are necessary

Experiences during maintainance dosage even with moderate daily doses used on a large number of patients have shown however that serious complications are not infrequent (5 12 13) Out of 230 patients in our department, who were given a dose equal to 1 2 g quinidine sulphate per day, serious quinidine syncope was observed in 8 cases (3) ECG recordings showed recurrent attacks of ventricular fibrillation lasting for several hours in 3 of these cases

There is a definite relationship between the effect of quinidine and the concentration of quinidine in serum (6 10 16 17) The incidence of serious complications increases rapidly with increasing concentration (2 4 6 10, 17) When quinidine sulphate is given 4 times per day considerable fluctuations in quinidine concentration are observed (9) Delayed absorption quinidine tablets were therefore introduced to avoid the peak levels — and the consequent increased danger of serious complications on the one hand — and the lowest values — with the following unsatisfactory effect on the other hand Long acting preparations also have the advantage that the number of doses per day can be reduced

In Norway 2 delayed absorption quinidine preparations are on sale In 1954 Systodin was introduced (8) Its

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blood vessels, several blood samples for DAO estimation have been obtained during the first minutes after rapid intravenous injection of heparin Our findings at present suggest that the thoracic duct does not mediate the DAO rise in the first phase nor do any *other organs that give their blood to the* superior vena cava The lungs do not seem to contribute materially to the early activity of this enzyme in plasma The intestinal mucosa, the liver and the kidneys have been shown to be relatively DAO rich organs in man (9) *Hitherto, our observations conform with* the assumption that the main source of the initial DAO increase lies in these organs

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Summary

The effect of parenteral doses of heparin on the plasma diamine oxidase (histaminase) activity was studied in healthy adults A large and rapid increase of the enzyme activity occurred after intravenous heparin (5 to 15 thousand units) This effect is similar to that previously found for lipoprotein lipase, but the plasma concentration followed a different time course in our subjects *Observations on thoracic duct lymph* indicate that it is an important transporter of the diamine oxidase to the blood plasma

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TABLE I The quinidine concentrations in serum (in mg/l) in 10 patients on maintamance dosage first with Kinidin Duretter for 5 days and then with Systodin the following 3 days Blood samples were withdrawn at 8 12 and 15 hours on the fifth day and the eighth day

	Kinidin Duretter			Systodin		
	Fifth day at			Eighth day at		
Pat no	8	12	15	8	12	15
4	3 30	4 40	4 35	4 45	3 50	6 10
5	3 40	5 40	5 00	3 80	3 90	4 20
6	3 50	2 40	2 00	3 70	2 00	1 40
11	2 80	6 70	7 65	2 70	3 20	6 15
12	3 60	5 00	4 00	3 60	2 60	2 00
13	0 60	3 10	2 30	2 90	2 40	1 90
14	3 30	5 90	5 50	6 90	5 40	4 60
15	4 40	7 85	6 00	6 50	5 15	5 50
16	3 50	3 60	4 80	3 90	3 40	5 15
20	5 90	7 70	6 25	4 60	7 00	5 20
Mean	3 43	5 41	4 79	4 31	3 86	4 22
Min	0 60	2 40	2 00	2 70	2 00	1 40
Max	5 90	7 85	7 65	6 90	7 00	6 15

TABLE II The quinidine concentration in serum (in mg/l) in 10 patients on maintamance dosage first with Systodin for 5 days and then with Kinidin Duretter the following 3 days Blood samples were withdrawn at 8 12 and 15 hours on the fifth day and the eighth day

	Systodin			Kinidin Duretter		
	Fifth day at			Eighth day at		
Pat no	8	12	15	8	12	15
1	2 50	1 60	3 50	1 60	2 60	2 90
2	3 80	5 75	4 25	3 05	3 95	3 40
3	3 50	3 90	4 20	2 60	3 45	4 30
7	4 60	3 80	3 05	3 75	4 20	4 20
8	3 80	5 70	5 50	4 40	4 40	4 60
9	4 30	3 10	2 40	3 05	3 60	3 40
10	3 80	3 90	4 30	3 20	5 00	5 45
17	4 20	3 50	2 40	2 90	3 20	3 70
18	3 00	5 30	3 45	2 30	3 90	3 45
19	4 30	5 15	5 20	5 10	7 60	7 60
Mean	3 78	4 12	3 83	3 20	4 19	4 30
Min	2 50	1 60	2 40	1 60	2 60	2 90
Max	4 60	5 75	5 20	5 10	7 60	7 60

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From Medical Department B and Cardiological Laboratory University Hospital Rikshospitalet Oslo Norway

Ventricular Septal Defect

A Clinical and Hemodynamic Study in 100 Cases

By

OLE STORSTEIN and SVEIN SORLAND

Isolated ventricular septal defect is one of the most common cardiac anomalies In our own material of 1 000 cases of congenital heart disease (13) ventricular septal defect and atrial septal defect were the most common anomalies each of them being encountered in 20 4 per cent of the total material Hemodynamic studies of later years and advances in cardiac surgery have served to clarify the anatomy and natural history in ventricular septal defect We now know that the previous common classification in two types Maladie de Roger and Eisenmenger complex does not cover the whole picture of this anomaly There is a whole spectrum from the small defects with no effect on cardiac hemodynamics to the most severe defects with reversed shunts and permanent cyanosis It has further been shown that the size of the defect is more important than its site (9) The division into membranous and muscular defect is no longer tenable Nor is the position of the aorta the determining factor to the cyanosis in large defects (7) The direction of the shunt in these cases is determined by the relationship between the resistances in the pulmonary and systemic circulations Hemodynamic studies have further demonstrated the spontaneous closure of small ventricular septal defects (1, 5 7), an encounter which was previously suggested but never proved

There are, however still unsolved problems in ventricular septal defect the frequency of small defects the frequency of bacterial endocarditis the indications for cardiac surgery With these problems in mind we are here presenting the first 100 cases of ventricular septal defect studied at the Cardiological Laboratory University Hospital Oslo

Material and clinical findings

Table I shows age and sex distribution in this material There was an equal number of men and women The majority of patients were below 20 years of age Only 2 patients

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TABLE I One hundred patients with ventricular septal defect

Age (yrs)	♂	♀
0– 9	34	22
10–19	12	17
20–29	6	4
30–39	1	2
40–49	2	0
	55	45

TABLE II Symptoms in 100 patients with ventricular septal defect

No symptoms	30
Dyspnea	53
Frequent respiratory infections	23
Poor weight gain	14
Cyanosis	15
Palpitations	11
Chest pains	5
Syncope	3
Squatting	2
Endocarditis	1

TABLE III Murmurs in 100 patients with ventricular septal defect

Grade	Syst murmur
1	1
2	2
3	11
4	39
5	39
6	8

were more than 40 years, the eldest being 46 years old A comparison with our material of 100 cases of atrial septal defect (12) shows that patients with ventricular septal defect (VSD) are admitted to the hospital at an earlier age than atrial septal defect (ASD), as an expression of the fact that VSD is a more severe anomaly than ASD and that the clinical findings are more prominent in VSD Fifty six patients were under 10 years of age as against 35 patients with ASD On the other hand only 2 patients with VSD were more than 40 years of age against 11 patients with ASD

Table II shows that 30 of the patients had no symptoms The most common complaints were dyspnea frequent respiratory infections and poor weight gain during the first year of life Cyanosis was found in *15 patients with VSD as against 6 patients* with ASD Bacterial endocarditis was found in 1 of the patients

Table III shows the intensity of the systolic murmur In most cases the strength varied between grade 3 and 5 The maximal intensity of the murmur usually was found in the 3rd and 4th left interspace but in a few cases it was found in the 2nd left interspace, making the differential diagnosis from pulmonic stenosis difficult On phonocardiography the systolic murmur in ventricular septal defect is characteristic being holosystolic and spoolshaped (fig 1) Usually there is an accentuated 2nd pulmonic sound which may be inconstant split It was earlier thought that the strength of the murmur depended on the size of the defect, a small defect giving a strong murmur and a large defect giving a weak murmur We have therefore in fig 2 compared the strength of the murmur to the size of the shunt As we see there is no close correlation a strong murmur may be found both in small and large shunts while a weak murmur is most commonly found in small shunts

When, however we take into consideration both the size and direction of the shunt and the pressure gradient across the defect we find that a weak murmur is usually found in patients with severe pulmonary hypertension and reversed shunt A strong murmur is found both in small defects with a small shunt and a low pressure gradient and in cases with a large shunt and high pressure in the right ventricle This is at

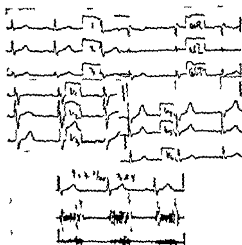


Fig 1 Phonocardiogram of the holosystolic murmur in ventricular septal defect

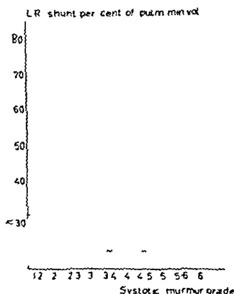


Fig 2 Relationship between strength of the systolic murmur and size of the left-to-right shunt

variance with Feruig et al s study (3) who found a poor correlation between the intensity of the murmur and the pressure gradient across the defect

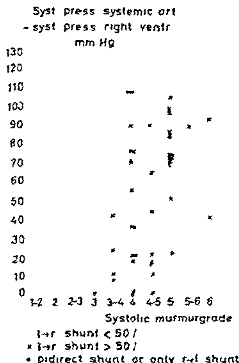


Fig 3 Relationship between size and direction of the shunt pressure gradient across the ventricular septal defect and strength of the systolic murmur

A diastolic murmur at the apex was found in 2J patients as an indication of relative mitral stenosis due to the high flow through the mitral valve

Electrocardiography

The ECG in VSD is characteristic demonstrating a so-called diastolic overloading of the left ventricle (fig 4) with high R waves in leads 2 and 3 and left precordial leads and with a small Q wave in the same leads In cases with pulmonary hypertension there is at the same time a high R wave in right precordical leads and inverted T waves in the same leads demonstrating systolic overload of the right ventricle (fig 5) The ECG may thus serve to evaluate the hemodynamics in VSD although in Schrire et al s 9 experience there was a

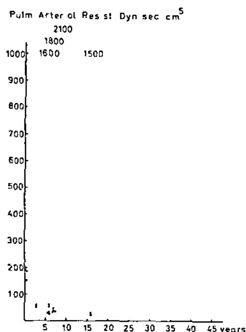


Fig 8 Relationship between pulmonary arteriolar resistance and age of the patients

weight gain and many of them die from heart failure or pneumonia Those who survive the first year of life run a serious risk of developing the Eisenmenger complex (2 5, 6) We therefore feel that palliative surgery should be done at an early age in these patients and that the banding' procedure (6) should be carried out with a view to corrective surgery when these patients have reached the age of 6—9 years

The surgical risk in Eisenmenger complex is so great that these patients should not be operated on Closure of the defect in these cases will place a heavy burden on an already heavily taxed right ventricle in instances of increasing pulmonary and right ventricular pressure The safety valve of the septal defect is removed by operation and the patients will develop right ventricular failure and die

In fig 8 we have compared pulmonary arteriolar resistance to the age of the patients As we see most of the patients have a low pulmonary arteriolar resistance, demonstrating the fact that the pulmonary vessels in most of these patients are distensible and able to accomodate a large increase in pulmonary blood flow without or with only slight rise in pulmonary artery pres ure Twenty six of the patients had pulmonary arteriolar resistance of more than 200 dynes, as against only 12 patients in our material of atrial septal defect (12) We see that most of the patients were of the younger age group, but that all patients up to 46 years were represented The age distribution in Bloomfield s series was different as in his material there was an accumulation of cases with severely increased pulmonary arteriolar resistance beyond the age of 15 years

So far repeated hemodynamic studies have not been carried out in this material The present presentation serves as a basis for future follow up which ought to be done in this patient group A further clarification of the natural history of this anomaly will serve to give more distinct indications for cardiac surgery than those presently prevalent

Summary

One hundred cases of isolated ventricular septal defect have been studied from a clinical and hemodynamic viewpoint and compared with atrial septal defect Ventricular septal defect presents as a more severe anomaly with earlier appear

ance of symptoms and with more marked disability There is a whole spectrum of clinical and hemodynamic findings from the small defects with no hemodynamic consequence to the most severe defects with severe pulmonary hypertension and reversed shunt

Surgical intervention is not indicated in the small defects and it is contraindicated in cases with reversed shunt Surgery should be considered in large defects, the operative risk in this patient group being 5 per cent at the present time The importance of carrying out banding procedure in infants with large ventricular septal defects is stressed

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From Medical Department B Medical Department F and Pathological Department Glostrup Hospital Glostrup and Medical Department B Gentofte Hospital Hellerup Denmark

Liver Histology in Ulcerative Colitis

By

Poul Anthonisen Per Christoffersen Povl Riis, Kay Schourup and Michael Schwartz

The liver disease which occasionally accompanies haemorrhagic non specific proctocolitis (haemorrhagic proctitis and ulcerative colitis) constitutes a problem which has been the object of several investigations based on clinical as well as biopsy and autopsy materials These studies hardly leave any doubt that there is often a pathogenetic relationship between the hepatic and colonic processes, but a deeper understanding of this relationship is still lacking

Two main possibilities seem to exist the liver disease can by considered either as a complication of the colitis or as a manifestation of the underlying pathological process parallel to the colonic inflammation

The present investigation was intended firstly to estimate the frequency of liver involvement in a series of patients with proctocolitis representing the full clinical spectrum of the disease and secondly to elucidate the nature of the pathogenetic relationship

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Methods and material

Primary material

Percutaneous liver biopsy using Menghini s technique was performed in 55 of the patients with verified haemorrhagic proctocolitis who were admitted to the County Hospitals in Gentofte and Glostrup during the period from April 1962 to June 1964 Thirty nine were women aged 15 to 70 years (average 38 7 years) and 16 were men aged 18 to 83 years (average 37 8 years)

The extension of the disease in the intestine had been determined by sigmoidoscopy and radiological examination of the colon in most cases using Welin s double contrast technique (in four patients colectomy had been performed) Table I shows the composition of the material with regard to extension of the disease

For the classification of the primary material with respect to the severity of proctocolitis at the time of liver biopsy the authors made use of Edwards and Truelove s criteria (5) (severe moderately severe mild) and added the grade inactive which implies that the patients had two or less movements daily no discharge of blood or pus and no abdominal discomfort (table II) The two patients in whom subtotal

TABLE I Extension of proctocolitis in primary material

	No of pats
Rectum	22
Rectum + sigmoid colon	12
Rectum + sigmoid and descending colon	5
Rectum + sigmoid descending and transverse colon	6
Whole colon	5
Whole colon + distal ileum	1
Total proctocolectomy performed	2
Subtotal colectomy performed	1
Subtotal colectomy + ileorectal anastomosis performed	1
Total	55

TABLE III Type of course of proctocolitis in primary material

	No of pats
Chronic continuous	10
Chronic intermittent	25
First attack	9
Less than two years duration	7
Total proctocolectomy performed (chronic continuous)	2
Subtotal colectomy performed (chronic intermittent)	1
Subtotal colectomy + ileorectal anastomosis performed (chronic intermittent)	1
Total	55

TABLE II Severity of proctocolitis in primary material

	No of pats
Inactive	3
Mild	15
Moderate	31
Severe	2
Total proctocolectomy performed	2
Subtotal colectomy performed — active inflammation in rectum	1
Subtotal colectomy + ileorectal anastomosis performed — active inflammation in rectum	1
Total	55

colectomy had been performed had active inflammation in their rectal stumps

The composition of the primary material with regard to type of course of the colon disease appears from table III In some cases liver biopsy was performed during first attacks, in others the duration of protocolitis was less than two years hence the course of disease in these 16 cases in all could not be classified at the time of examination Subsequent observation showed, however that ten of these patients had the chronic continuous, and six the chronic intermittent type of proctocolitis For the patients in whom colectomy had been performed the type of course before the operation is indicated For the remaining patients the average duration of proctocolitis at the time of liver biopsy was 9 2 years In 14 patients the disease had lasted ten years or more, the longest duration being 35 years The four patients in whom colectomy had been performed had had their proctocolitis for four, 11 14 and 15 years at the time of operation which had been carried out four months seven one and three years respectively before the liver biopsy

The liver status of the patients at the time of examination was given by the following blood analyses serum bilirubin (in some cases icterus index) prothrombin proconvertin index alkaline serum phosphatase glutamate pyruvate transaminase serum electrophoresis and in some patients also thymol turbidity and zinc sulphate test In a few of the patients one or more of these examinations were not carried out but in only two



Fig 1 Liver biopsy from proctocolitis patient Small round cell infiltrate Haematoxylin-eosin Appr × 225



Fig 2 Liver biopsy from proctocolitis patient Large round cell infiltrate Haematoxylin eosin Appr × 225

none of them were performed making an evaluation impossible

Systemic glucocorticoid treatment was given to three patients at the time of investigation local glucocorticoid therapy (enemas) to 23 patients including two of the three just mentioned

The microscopical examination of the *primary material of liver biopsies was undertaken by the two pathologists on the team* along the following lines the 55 biopsies were first evaluated independently by the two examiners They both soon found that three changes appeared frequently namely round-cell infiltrates (figs 1 and 2) hyperpigmentation of the liver cells and Kupffer cell hyperplasia *During* subsequent studies of the preparations it was however realized that the latter two changes were difficult to define so that the ultimate criterion of pathological histology was determined as the presence of inflammatory cell infiltrates ("round cells") in the liver parenchyma and or the portobiliary spaces

When the primary evaluations of the two examiners were compared they showed agreement *in 42 cases The remaining 13 biopsies* were reviewed independently by the examiners after which agreement was obtained in 12 cases

Recovery material

To find out if the pathological changes which were found in some of the liver biopsies from the primary material were specific to haemorrhagic proctocolitis an examination was made of all liver biopsies sampled at Glostrup Hospital during the time when the primary material had been collected including the latter A total of 259 biopsies were examined along exactly the same lines as those described above for the primary material concerning both criterion of pathological histology and independent evaluation by *two pathologists*

In 228 cases the judgments of the examiners agreed primarily The remaining 31 biopsies were reviewed and discussed whereafter agreement was obtained in most cases Both pathologists maintained their conflicting views in a few preparations none of which however originated from patients with proctocolitis

The examination of the primary as well as the recovery material was performed blind ly The two pathologists had no knowledge of the clinical data of the patients from whom the biopsies derived

Results

Estimated by the blood analyses mentioned above only one patient from the primary material had unequivocal liver damage while the analyses in the greater part of the remaining showed normal conditions in few cases questionable changes The biochemical signs of liver damage were shown by the patient in whom total proctocolectomy had been

TABLE IV Main features of results of present investigation (for further explanation see text)

Primary material — 55 liver biopsies	22 pathological	Criterion of pathological histology round cell infiltrates (C)
	33 normal	
Recovery material —259 liver biopsies	54 positive with regard to C	19 from proctocolitis patients (22−4+1)
		35 from other patients
	205 negative with regard to C	36 from proctocolitis patients (33+4−1)
		169 from other patients

performed seven years before the time of biopsy

The main features of the histological studies appear from table IV

Pathological findings were made in 22 of the liver biopsies from the *primary material* On the whole the changes were moderate being very mild in more than half the cases and only pronounced in three The positive findings consisted of cell infiltrates in the liver parenchyma and or the portobiliary spaces, irrespective of size and number Most of the cells looked like lymphocytes, but there were also a few somewhat larger cells — probably proliferating Kupffercells — and occasional eosinophilic granulocytes The infiltrates varied in size from quite small — consisting of from five to six cells — to rather large (figs 1 and 2) Necrotic liver cells in the circumference of infiltrates in the parenchyma were not demonstrated with certainty Fatty degeneration was found in four cases in three of these the changes were slight, while in the fourth the finding was surely pathological but did not include degeneration of cell nuclei or incipient cirrhosis On the whole cirrhosis was not seen in one single biopsy, nor were intrahepatic cholestasis or signs of infectious hepatitis encountered

The demonstration of cellular infiltrates in the liver biopsies showed no correlation to the age or sex of the patients to the extension of the process in the intestine, to the degree of activity of the disease and to the length of history at the time of biopsy, or to the type of course This lacking correlation applies to the factors singly and combined

Among the 259 liver biopsies from the *recovery material* 54 satisfied the established criterion of pathological histology the finding of cellular infiltrates When the code was broken it appeared that among these 54 biopsies were 18 of the 22 from the primary material which had been considered pathological at the first examination the four biopsies which were not recovered had only showed very mild changes primarily In addition one biopsy from the primary material was found which had been judged normal at the first examina

tion, here, too very mild changes were present

The remaining 35 preparations of the recovery material which were positive with regard to the established criterion originated from patients with a variety of diseases

In only four cases were the biopsies essential for the diagnosis in the rest of the patients the diagnoses were made by other means or they remained obscure Eleven patients had well-defined primary liver and gall bladder disease while two had liver changes in connection with other diseases (infectious mononucleosis and pancreatic carcinoma with liver metastases) In six cases liver biopsy was performed as part of examination for generalized connective tissue disease in only one of these was this diagnosis confirmed with certainty (polyarteritis nodosa) One patient had Hodgkin s disease In the remaining 15 patients liver biopsy was carried out on various indications as e g fever of unknown origin unexplained hepatomegaly light jaundice raised serum alkaline phosphatase or hypoalbuminaemia of unknown cause in this group the diagnoses in three cases proved quite different from those expected (hypernephroma mediastinal reticulosarcoma congestive heart failure) while the diagnoses in the rest of the cases remained unsolved

Thus the changes which had been demonstrated in 34 of the liver biopsies from the recovery material were not characteristic of any special disease Therefore an assessment was made of these patients histories in an effort to find common factors which were possibly responsible for the liver lesions in patients with as well as patients without, proctocolitis As it appears from table V this attempt to demonstrate a connection was also futile The factors investigated were the occurrence of hepatitis or other liver/gall bladder diseases at an earlier date a history of alcoholism the presence of cachexia or severe emaciation at the time of liver biopsy and the performance of radiological examination of the colon within three months before the biopsy The double-contrast technique of Welin always and the traditional technique sometimes includes the use of tannic acid containing enemas)

Table V also shows the extent of corticosteroid therapy in the two groups Naturally such therapy would rather tend to moderate any inflammatory liver process

TABLE V Occurrence of some relevant factors in patients with liver changes — with and without proctocolitis

	Pats with proctocolitis	Pats without proctocolitis
Liver/gall bladder disease	1	8
Alcoholism	1	5
Cachexia	0	4
Double contrast examination	[1]9	0
Barium enema examination	[2]7	6
Systemic glucocorticoid therapy	2	5
Local glucocorticoid therapy	[3]8	1

[1] Including the patient with alcoholism
[2] Including two patients also examined with double contrast technique
[3] Including two patients in systemic therapy

progresses after colectomy, and it also seems to be strengthened by immunological studies from recent years (3, 4, 7, 8)

On the other hand the preponderance of other extracolonic processes in our group of proctocolitis patients without liver changes does not suggest that these changes are manifestations, because the various extracolonic processes are often multiple in patients with protocolitis

The question of complication versus manifestation can thus not be answered unequivocally on the basis of the present investigation Consequently the presence of mild or moderate hepatic lesions should not, as a rule, influence the indication for proctocolectomy in a given patient

Summary

In 55 patients with verified haemorrhagic proctocolitis (haemorrhagic proctitis and ulcerative colitis) representing a large clinical spectrum of the disease including very light cases percutaneous liver biopsy was performed (the *primary material*)

The biopsies were assessed independently by two pathologists who had no knowledge of the clinical data In 22 preparations large or small cellular infiltrates, consisting mostly of lymphocytes, were found in the portobiliary spaces and/or the liver parenchyma, and in four cases there was fatty degeneration which was only pronounced in one None of the biopsies showed intrahepatic cholestasis, cirrhosis or hepatitis like changes The mere presence of cellular infiltrates was then chosen as criterion of pathological liver histology

A *recovery material* was examined along the same lines, consisting of all liver biopsies (259) obtained at the Glostrup Hospital during the time when the primary material had been collected, including the latter In 54 biopsies from the recovery material 'round cell" infiltrates were found Eighteen of the liver biopsies from proctocolitis patients which had primarily been considered pathological were recovered while four were missed One further proctocolitis biopsy, primarily estimated as normal, was now considered pathological The remaining biopsies in the recovery material which contained round cell infiltrates originated from patients with a variety of diseases including primary liver/gall bladder disease, infectious mononucleosis, polyarteritis nodosa, carcinoma of the pancreas, and Hodgkin's disease

No pathogenetic factors (earlier liver/gall bladder disease, alcoholism, cachexia, tannic acid enemas) were common for patients — with and without proctocolitis — from the recovery material whose liver biopsies showed pathological changes, nor was any correlation demonstrated between the hepatic lesions of the patients with proctocolitis and the extension, degree of activity, duration, or type of course of the intestinal disease

The two groups of proctocolitis patients — with and without liver changes — were also compared with regard to the pathogenetic factors mentioned above plus another two the administration of blood or hepatotoxic agents

within six months before the biopsy The comparison did not support the conception that the sum of these factors could be responsible for the hepatic lesions

The lack of correlation between the liver changes and the extension duration, or severity of the proctocolitis speaks against the view that the liver disease is a *complication* of the intestinal inflammation On the other hand the preponderance of other extracolonic processes in the group of proctocolitis patients *without* liver lesions does not favour the conception that the liver changes are *manifestations* parallel to the colitis

Thus, the presence of mild or moderate hepatic lesions should not, as a rule strengthen the indication for proctocolectomy in a given patient

Acknowledgement

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Addendum

After completion of the present work an up to-date survey has been given by S Mistilis and S Goulston in Recent advances in gastroenterology Churchill London 1965

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From the Central Laboratory (Head P Paaby M D) Aalborg County Hospital, Aalborg Danmark and the Skørping Sanatorium (Head E Strandgaard M D) Skørping Danmark

The Absorption of Vitamin B_{12} During Treatment with Para-aminosalicylic Acid

By

P Paaby and E Norvin

It has been reported, that treatment with Para aminosalicylic acid (PAS) can be the cause of various haematological complications including megaloblastic anaemia and in 1964 Heinivaara and Palva (3) published a report on the effect of PAS treatment on the Schilling test

They reported a significant fall in the Schilling test values with or without intrinsic factor within a few weeks after the institution of PAS treatment, the values returning to pretreatment levels a few weeks after discontinuation of the medication The most striking feature of this publication was that all of 10 cases responded so definitely to the PAS treatment with rapid falls in Schilling values

We have tried to evaluate the influence of PAS treatment upon the Schilling test and various other parameters in a series of 26 patients

Methods

Schilling test (7) Normal values 11—35 % (1 Each test dose of vitamin B_{12} CO^{58} contained 1 µg vitamin B_{12} and the flushing dose was 1 000 µg The amount of intrinsic factor was 100 mg freeze dried hog pyloric mucosa (Biofac) Analysis of the concentration of vitamin B_{12} in serum (serum B_{12}) was carried out by the Central Laboratory Central Hospital Randers by the method of E Hoff Jørgensen (4) Normal values 150—800 pg/ml (All other analyses were performed in the Central Laboratory Aalborg County Hospital) Concentration of iron and transferrin in serum by Ramsay s (6) method Normal values serum iron 46—154 µg% serum transferrin 245—405 µg% (5) Haemoglobin was determined as cyanmethaemoglobin (2) Normal values ♂ 14—17 g% ♀ 12—15 g% (5) Haematocrit value was determined by Wintrobe s method and erythrocytes counted mechanically on the Celloscope

Mean cell volume (MCV) was calculated as

$$MCV = \frac{\text{Haematocrit} \quad 10}{\text{red cell count mill}/\mu l}$$

Normal range 86—107 mµ³ (5)

Mean cell haemoglobin concentration (MCHC) was calculated as

$$MCH = \frac{\text{Haemoglobin g\%} \quad 100}{\text{Haematocrit}}$$

Normal range 32—36 g/100 ml (5)

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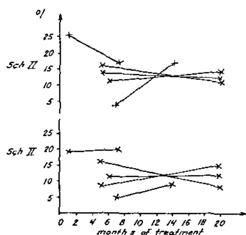


Fig 1 Schilling values without (Sch I) and with (Sch II) intrinsic factor in five patients examined twice during treatment with para aminosalicylic acid (PAS)

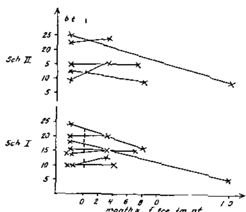


Fig 2 Schilling values before (b t) and during treatment with PAS

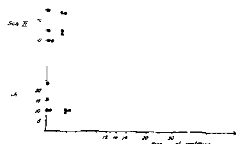


Fig 3 Schilling values in 26 patients during treatment with PAS

Patient material and treatment

Twenty six patients Fourteen were ♂, 25—71 years of age Twenty two were ♀ 28—78 years of age Five ♂ and 7 ♀ were investigated twice Two ♂ and 0♀ received 3 g phenyl PAS + 1 g PAS 3 times daily No ♂ and 4 ♀ received 4 g phenyl PAS 3 times daily Twelve ♂ and 8 ♀ received 9 g Ca PAS once daily

Results

No differences could be found between patients treated with Ca PAS and those treated with Phenyl PAS or Phenyl PAS + PAS Statistically they are therefore treated as belonging to the same group

Fig 1 shows the relationship between duration of treatment and Schilling test without intrinsic factor (Sch I) and with intrinsic factor (Sch II) in five patients examined twice *No statistical* difference could be shown between the groups of patients examined 1st and 2nd time

Fig 2 indicates changes in Sch I (7 patients) and II (5 patients) before and after commencement of treatment (One patient, who had been treated during 120 months was first examined immediately before cessation of treatment and then again after 5 months without PAS)

No statistical difference in Schilling values could be shown between the groups of untreated and treated patients, but it may be noted that the two patients whose Schilling values dropped were those who had had the longest periods of treatment (8 and 120 months respectively)

Fig 3 shows Sch I and II in *all the* patients investigated Regression anal

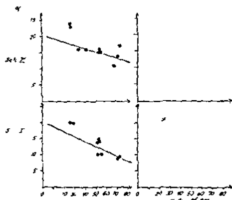


Fig 4 Relationship between age and Schilling values Females = ● males = Y

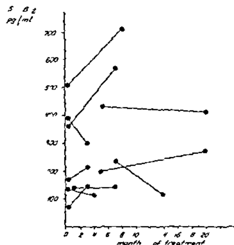


Fig 5 Serum concentration of vitamin B_{12} (Se B_{12} pg/ml) in 10 patients examined twice during treatment with PAS

ysis was carried out (When a patient had been examined twice, only the last set of results was used in the calculation)

No relationship between Schilling values and duration of treatment could be shown Many results were below the normal range and there was a feeling that older women predominated among patients with low results

Fig 4 shows the regression lines calculated for relationships between age and Schilling values in female patients The regression coefficients were not significant There was no relation between Schilling values and age of the male patients, but fig 4 indicates the critical point of the Schilling test in routine work the collection of 24 hour urine specimens

Fig 5 shows serum B_{12} in 10 patients examined twice during treatment, and fig 6 shows serum B_{12} values in all the

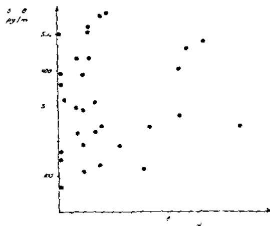


Fig 6 Vitamin B_{12} in serum (pg/ml) versus duration of treatment with PAS

patients There was no relationship (regression analysis) between serum B_{12} values and the duration of treatment

The statistician also tested the possibility of relationship between serum B_{12} and Sch I and II, duration of treatment and Hb, MCV, MCHC, serum iron, transferrin, but none of them were significant

He found, though a strongly significant relationship between Sch I and Sch II This in itself is quite trivial but from an analytical point of view it is satisfactory When as in this material the administration of intrinsic factor is unnecessary Sch I and Sch II should give identical results If not, the technical work is bad

Since the patient material consists of seriously ill persons (TB) it is not surprising that so many of the results fall outside the normal ranges

Summary

In 26 patients treated with paraamino salicylic acid the possibility of relationship between duration of treatment and Schillingtest values, concentration of vitamin B_{12} in serum, concentration of iron and transferrin in serum, concentration of haemoglobin in whole blood, mean cell volume, and mean cell haemoglobin concentration were tested

No such relationships could be proved, and the results of Heinivaara and Palva (3) could not be corroborated

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From the Department of Dermatology University of Uppsala Uppsala, and the Department of Internal Medicine Central Hospital Eskilstuna Sweden

Necrotizing Angiitis with Multiple Widespread Hemorrhagic Infarctive Lesions of the Skin

By

O Groth, C-O Lindemark and S G Sjöberg

Drugs and infections play an important part in eliciting necrotizing processes in the skin but the mechanism is as yet unkown When the cutaneous target is superficial, it can lead to cleavage in an epidermal plane and give the clinical picture of "toxic epidermal necrolysis (7, 19) When deeper necroses of the skin are reported, alterations in small or medium sized vessels are often observed A group of vascular disorders characterized by inflammatory reactions and fibrinoid changes of the vessel wall, is usually called necrotizing angiitis (13 18 20)

In the fatal case described here a rapidly developing skin rash led to a hemorrhagic infarctive process followed by gangrene over several extremely large circumscribed areas of the body Onset was associated with a cold or influenza for which the patient had received a combination of sulphonamides and penicillin Post mortem findings revealed a disseminated fibrinoid angiopathy

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Case report

A 44 year-old house painter was admitted to hospital on June 15 1963 Six days previously he had complained of a sore throat especially on swallowing and head ache and his temperature rose to 39—40 C. Several similar episodes in recent years had been sucessfully treated with sulphonamides and penicillin No complications of medica tion were then observed On the following day treatment with a combined pencillin-sulphonamide preparation was begun (phe noxipropyl pencillin sulphadiazine sul phamerazine and sulphadimidine) The temperature and other symptoms were not influenced however and he complained of pains all over the body

Two days before admission to hospital the patient stated that his skin felt thick stiff and swollen The medication was stopped partly because it was obviously ineffectual and partly because of the patient s cutaneous symptoms Tetracycline hexametaphosphate was substituted About 10 hours later large areas of the swollen skin were becoming in creasingly deep red The pain was more and more localized to the affected areas of skin and receded only after taking an analgesic preparation (salicylic acid phenacetin coffe

ine, paracetanolum) By the 15th of June the affected skin area was greatly increased In addition it had partly taken on a blue black color and the pain was excruciating

On admission to the hospital on the afternoon of June 15th large areas of skin on both the front and back of the torso the thighs buttocks and the upper arms had an ab normal blue black coloring with a narrow red marginal zone The line between the normal and the abnormal skin was sharp The lesions were intensely infiltrated and the outline of these areas were slightly elevated Similar changes in the skin of the forearms and legs as well as the cheeks and lobes of the ears could be seen but the changes were not so extreme Bleeding was seen in the mouth mainly in the palate A tendency to increase and confluence to larger skin lesions came about in the following hours but the spread then stopped (steroid effect?) During the following days a strong tendency to vesiculation occurred and large bullae with partly hemorrhagic content had developed in the blue black areas of skin which eroded and began to drain successively After the epidermis was denuded in this manner the abnormal blue black infarcted cutis and subcutis began to necrotize and denude during the following weeks (see figs Although the patient s general con dition on admission was poor it slowly worsened as this process continued His temperature remained quite constant around 38 —38 5 C and his pulse rate about 80 The B P was normal He could be fed orally the whole time

Laboratory data The admission laboratory examinations (Brante) revealed a Hb of 86 (13 15 g/100 ml) red cell count 4 4 mill mm³ white cell count 2 500/mm³ with 35 % segmented neutrophils 27 % band forms 0 % eosinophils 0 % basophils 26 % lymphocytes and 6 % monocytes Sternal puncture (Brante) revealed a bone marrow with normal cellular constituents The ESR was 30 mm in the first hour The platelet count was 110 000/mm³ The whole blood coagulation and the skin bleeding times were within normal values and there was no abnormal clot retraction Plasma fibrinogen value and plasma fibrinolysins were normal Prothrombin time (Quick) was 68 % The total serum bilirubin was 0 62 mg % with positive indirect reaction (van der Bergh) the alkaline phosphatase 6 7 Thymol turbidity test 11 1 units Electrophoresis was unrevealing Blood urea nitrogen was normal

Anemia gradually developed during the following days On June 20th the Hb value was 61 %, red blood count 3 09 mill /mm³, white cell count 14,200 with 60 % segmented neutrophils 4 % band forms 28 % lymphocytes 5 % monocytes 1 % eosinophils and 0 % basophils Platelets numbered 412 000 Thymol turbidity decreased to 9 6 but alkaline phosphatase increased to 31 8 The blood values tended to become normal or subnormal with blood transfusions and on June 29th were Hb 69 % red cell count 3 51 mill /mm³, white cell count 12 400 polynuclears 9 600 and mononuclear cells 2 800/mm³

Therapy On admission steroid therapy was given intravenously, 100 mg hydrocortisone sodiumsuccinate and at the same time 8 mg triamcinolone was prescribed orally four times daily The latter dose was decreased after a time Tetracyclinehexametaphosphate was given even during hospitalization

The patient s extreme pains raised a difficult problem The earlier used sali cylates as well as several analgesics were as a rule of little effect It proved necessary to use morphine and morphine like alkaloids in combination with chlorpromazine When extensive changes of dressings were made it often was necessary to do this under general anesthesia On these occasions parts of the necrotic tissue often were removed Since the condition reminded one of severe burns treatment to a large extent consisted of airing and dry protected bandage The pa tient was turned according to a certain sched ule throughout the day but as a result of the tremendous pain, this was gradually





2

3





4

Fig 1 Bullous infarctive lesions a few days after admission to hospital

Fig 2 The same area about three weeks later showing gangrene and denudation

Fig 3 Small vessel in the spleen with fibrinoid reaction (red) Stained according to Ladewig

Fig 4 Accumulation of PAS positive material in the kidney (Figs 3 and 4 by courtesy of B Ivemark)

changed to having the patient lying on his stomach respectively his back for a day at a time He was treated in isolation and all attending personnel carefully observed sterile precautions with clothing gloves and masks Several blood transfusions were given and the diet was made richer in protein Anabolic steroids were also given

Course A certain healing tendency was gradually observed in the edges of the ulcerations but the widespread gangrenous changes remained and extended now deep into parts of the muscle tissue As the patient s general condition gradually worsened it was judged necessary to try to remove the necrotic areas more radically and this was done by plastic surgery on July 18th and 20th After a preliminary improvement in connection with thi the patient died on July 22nd

The necropsy (B Ivemark) revealed a condition of sepsis and edema of the lungs The microscopic findings were consistent with hypersensitivity angiitis with secondary sepsis

Affected arterioles were found in the spleen kidney and liver The changes were most pronounced in the former organs The vessels showed necrosis of the wall with accumulation of PAS positive material which reacted as fibrinoid when stained according to Ladewig Around the arterioli many plasma cells and some immature cells of an unidentified nature were found There were no eosinophils Fibrinoid thrombi were also noted in some glomerular tufts (figs 3 and 4)

Focal necrotic areas outside the vessels were seen in the spleen kidney lung liver and lymph nodes Most of them were infiltrated with polymorphonuclear leukocytes and were interpreted as septic lesions Abundant growth of proteus and staphylococcus aureus was obtained from blood liver lung spleen and kidney

Additional findings Fat droplets in large numbers in liver parenchymal cells particularly in centrilobular regions Moderate non specific lymphadenitis with hyperplasia Septic splenitis

Comments

In this particular case the cutaneous manifestations were so conspicuous that they came to completely dominate the picture of this illness The widespread bullous-infarctive and intensive hemorrhagic processes in the skin were such that they could very possibly explain the anemia as well as the slightly pathological liver findings and also the occasionally appearing proteinuria and hematuria It is amazing that the disseminated and, in some organs prominent, necrotizing angiitis which could be substantiated by post mortem examination, did not present more symptoms during the initial period of the patient s illness It cannot be excluded that this visceral engagement at least in part could have developed during the six weeks in which the patient was kept alive and that the toxic elements which were probably released continuously into the blood and lymph system from the gangrenous skin could be an assisting factor It seems however most probable that it is a matter of a primary disseminated form of necrotizing angiitis and that even if representative skin sections could not be obtained the vessel changes were of the same type both in viscera and skin

A very large number of clinical syndromes present common morphological changes which are designated as necrotizing angiitis by Zeek 19) who uses it to encompass this entire group of lesions Leucocytoclastic angiitis and allergic angiitis (or vasculitis) are also used as the nearest synonomous concept (8, 9, 13 18) Several isolated cases as well as group case studies have been reported in recent years and additional

proposals have been presented for classification Usually, this classification has taken as a primary point the size of the affected vessel in small and average sized vessels, and its localization to the skin only or whether systemically distributed (9, 11, 13) For the present it appears that a simplified clinical morphological sub division as Winkelmann and Ditto (18) proposed is the most practical Cutaneous angiitis is classified into 1 papulo petechial, 2 bullous infarctive, and 3 chronic plaques forms Systemic angiitis is classified into 1 true periarteritis nodosa, 2 hypersensitivity angiitis (as defined by Zeek), and 3 granulomatous angiitis (including Wegener's and allergic granulomatosis) It is of the greatest importance to be aware that the cutaneous manifestations can only be a symptom of a primary systematic angiitis In addition there is no definite line between the different sub groups several cases are described as being of mixed forms With this classification it is advantageous to include, for example, periarteritis nodosa cutanea (5, 6, 8) the different symptom complexes of Gougerot, anaphylactoid purpura and other leukocytic hemorrhagic microbids (8 9 13)

Cases with such extensive and intensive hemorrhagic infarctic processes in the skin as in our patient are very seldom seen It seems however, most logical to classify our case among the group which Zeek lists under hypersensitivity angiitis' Zeek (20) writes 'As it is seen in man, hypersensitivity angiitis is an acute necrotizing inflammation in and around the smallest branches of the intrinsic blood vessels, both arterial and venous, of the viscera and connective tissues, including the dermis and, in advanced cases of the process, spreads to larger vessels" There are, however, cases reported with large hemorrhagic necroses of the skin similar to those of our patient, but histological investigations are lacking or fail to reveal any necrotizing angiitis Such cases are described during therapy with dicoumarol and its derivatives (2, 16) or under the descriptive heading "Acute idiopathic circumscribed gangrene (15) It is obvious that cases such as these create problems when using a classification based upon anatomical grounds

In discussing the etiology of necrotizing angiitis, drugs as well as bacterial sensitization (10, 14) have been particularly emphasized Preceding the vascular illness our patient had not only an infection but had also been treated with sulphonamides and penicillin for it, both of these have been mentioned as suspect causes

The pathogenesis is unknown, but some type of immune mechanism seems most probable (3) Besides an antigen antibody reaction or an autoimmune mechanism, toxic reactions have been emphasized It seems attractive however in cases like ours to see the infection as some type of preparative factor and the drug as the provocative or eliciting component (1 12) In this way the mechanism, like the pathological changes, would be consistent with Schwartzman Sanarelli reaction experimentally induced in animals

In the case reported here the spread of the hemorrhagic infarctive process in the skin stopped some hours after

cortisone treatment was started, and it was our impression that this therapy was of benefit (17) No improvement, however, could be seen in the already affected areas nor was the develop ment of gangrene or the denudation stopped The patient presented an insurmountable problem as a result of his extreme skin pain and tenderness and we were forced finally to carry out dressing under general anesthesia Swab tests from the necrotizing skin showed no pathological growth on culture in the beginning but, in spite of careful, sterile precautions, a surface infection developed with strains of staphylococci and proteus It was not surprising that once the infection had begun in the skin it developed into septicemia

Summary

A patient treated for symptoms of influenza with sulphonamides and penicillin developed extremely widespread hemorrhagic lesions of the skin, followed by gangrene Post mortem findings reveal ed visceral involvement with inflamma tory reaction and fibrinoid degeneration of the vessels walls It seems to us most logical to classify our case among the group listed as hypersensitivity angiitis (Zeek) probably caused by the combined effect of an infection and the therapy

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From King Gustaf V Research Institute (Head G Birke M D) and the Department of Internal Medicine Karolinska Hospital (Head H Lagerlof M D) Stockholm Sweden

Acute Effects of Nicotinic Acid on Plasma, Liver, Heart and Muscle Lipids

Nicotinic Acid in the Rat II

By

Lars A Carlson, Sven O Froberg and Edwin R Nye[1]

The inhibition of free fatty acid (FFA) mobilization from adipose tissue by nicotinic acid (3, 9) has been extensively confirmed and was recently reviewed (4 5) Nicotinic acid has been used as a tool to study the consequences of inhibition of FFA mobilization in the normal condition and in different pathological states (for review, see 4, 5) In this work the effect of nicotinic acid on local lipid pools in various tissues will be described

In the fasting state FFA is believed to be the major circulating metabolite for the supply of energy However, the administration of nicotinic acid to man which is followed by a prompt reduction of the levels and turnover of FFA does not reduce the oxygen consumption in normal subjects at rest (11, 15) or during exercise (6) or in the hyperthyroid state (4, 11) Although the FFA turnover was much lower than the total calorie consumption in these studies the RQ increased only slightly from values around 0 7 (6 15) or not at all (11) indicating that lipids were still the major substrate for oxidation This raises the possibility that local tissue lipid pools are utilized when the availability of FFA is so far lowered that tissue substrate requirements can no longer be supplied from this source Recently we have studied the acute metabolic consequences of inhibition of FFA mobilization in the rat upon plasma and liver triglycerides (TG) and cholesterol as well as blood glucose (8) It was shown that reduction of plasma FFA levels in the rat was rapidly followed by a fall in plasma and liver TG, in that order and also by reduction in plasma, but not in liver cholesterol Blood glucose was also reduced It was considered probable that the fall in plasma and liver TG was due to decreased liver TG synthesis consequent upon the reduction in available FFA

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[1] Present address Department of Medicine University of Otago Medical School Dunedin New Zealand

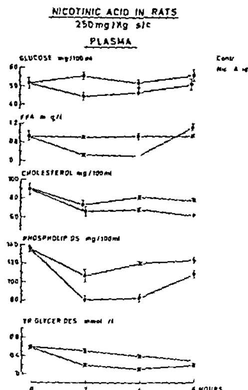


Fig 1 Mean values for glucose FFA cholesterol phospholipids and triglycerides in plasma of saline and nicotinic acid treated fasting rats Bars indicate SEM

If TG synthesis is reduced in the liver as an indirect consequence of the action of nicotinic acid then it would seem possible that TG pools in other tissues might also be affected possibly as a result of impaired synthesis following reduction in available FFA with a further possibility of a concomitant normal, or increased utilization of fatty acids stored in the intracellular TG pools as other respiratory substrates are depleted as result of the action of the nicotinic acid

The present study was directed therefore at following the lipid levels in cardiac and skeletal muscle, as well as in plasma and in the liver, during the first few hours following nicotinic acid administration in rats and the experimental design paralleled that of the previously reported investigation (8) A preliminary note on the acute effects of nicotinic acid on TG pools in heart and skeletal muscle has appeared (5)

Material and methods

Animal procedures Male Sprague Dawley strain rats (AB Anticimex Stockholm) weighing 180—200 g were used The animals were accustomed to the laboratory surroundings for 24 hours before use The nicotinic acid solution used consisted of a 5 % solution of the sodium salt and was injected subcutaneously Control animals were injected with an equivalent volume of a sterile solution of physiological saline The rats were not anaesthetized and moved freely in their cages The dose of nicotinic acid used was 50 mg per rat, equivalent to 250 mg/kg All rats were fasted for 18 to 20 hours before injection but drinking water was provided The experiments were started at about 9 a m

Control and test animals were sampled alternately and were bled by heart puncture after stunning by a blow on the head and the blood collected into heparinized syringes and stored in iced water Livers and hearts were immediately removed after bleeding and frozen in liquid nitrogen Both gastrocnemius muscles were exposed cleared of connective tissue removed and also frozen in liquid nitrogen Subsequent storage was at — 20 C Separation of the red and white muscle fibres of the gastrocnemius muscle was carried out after thawing of the tissues as described in detail by Froberg (12) Preparation of other tissues was carried out as previously described (8)

Biochemical determinations Plasma and tissue lipids were estimated as previously described (2 8 12) Plasma glucose was determined by the method of Marks (16) and plasma

TABLE I Values for FFA cholesterol phospholipids and triglycerides in plasma in fasted rats injected with saline (C) or nicotinic acid (N)

Time after injection		2 hours			4 hours			6 hours		
		C	N	P	C	N	P	C	N	P
FFA (mEq/l)	M	0.88	0.50		0.91	0.48		0.90	1.09	
	SEM	0.04	0.03	<0.001	0.05	0.02	<0.001	0.04	0.07	<0.05
	n	11	11		11	11		11	11	
Cholesterol (mg/100 ml)	M	72	65		80	67		76	60	
	SEM	5	5	>0.05	2	1	<0.001	7	2	<0.05
	n	11	11		11	11		11	11	
Phospholipids (mg/100 ml)	M	106	80		119	82		122	97	
	SEM	7	5	<0.01	2	4	<0.001	3	4	<0.001
	n	11	11		11	11		11	11	
Triglycerides (mmole/l)	M	0.48	0.18		0.38	0.10		0.37	0.18	
	SEM	0.04	0.02	<0.001	0.03	0.01	<0.001	0.04	0.02	<0.001
	n	11	11		11	11		11	11	

M=Mean value SEM=Standard error of the mean n=number
P indicates the statistical significance of the difference between controls and nicotinic acid treated rats

FFA by the Trout modification (20) of the Dole method (10)

Results

Blood

The levels of glucose, FFA, cholesterol and TG in plasma followed the same general pattern seen in the previous study with the same dose of nicotinic acid (fig 1 table I) The FFA levels were depressed at 2 and 4 hours after injection of nicotinic acid and slightly elevated after 6 hours Cholesterol and triglycerides in plasma were depressed in a fashion similar to the one described previously (8) The present study in addition shows that the concentration of phospholipids in plasma was significantly lowered at 2 4 and 6 hours in the nicotinic acid treated group

Liver

In the livers the TG content was significantly depressed at 2 and 4 hours in the rats given nicotinic acid while no changes had occurred in the cholesterol content between the two groups (table II, fig 2) These findings are consistent with the results of the previous study (8) Table II also shows that nicotinic acid had significantly reduced the level of phospholipids in the livers at all times

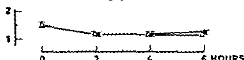
Heart

The amount of TG in the hearts was lower in the nicotinic acid treated group at all times (fig 3) but the effect was statistically significant only at 2 hours (table III) In an identical pilot study reported previously (5) it was found that the TG content of the hearts of saline

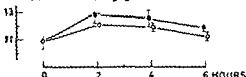
NICOTINIC ACID IN RATS
250mg/Kg s/c
WHITE MUSCLE

o Contols
• Nic Acid

CHOLESTEROL mg/g



PHOSPHOLIPIDS mg/g



TRIGLYCERIDES µmole/g

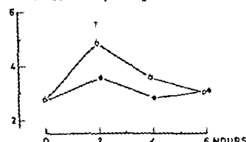
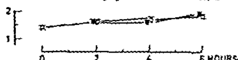


Fig 4 Mean values for cholesterol phospholipids and triglycerides in the white muscle part of the gastrocnemius muscle of saline and nicotinic acid treated fasting rats Bars indicate SEM

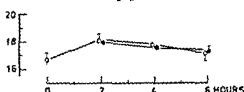
NICOTINIC ACID IN RATS
250mg/Kg s/c
RED MUSCLE

o Controls
• Nic - Acid

CHOLESTEROL mg/g



PHOSPHOLIPIDS mg/g



TRIGLYCERIDES µmole/g

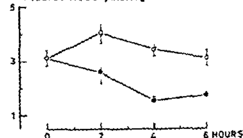


Fig 5 Mean values for cholesterol phospholipids and triglycerides in the red muscle part of the gastrocnemius muscle of saline and nicotinic acid treated fasting rats Bars indicate SEM

tent of white skeletal muscle (table IV fig 4) Not even in the red muscle with its characteristically high phospholipid content (12) were there any differences in the levels of cholesterol and phospholipids between the two groups (table V) The concentration of TG in red muscle was, however, significantly depressed at 2, 4 and 6 hours by nicotinic acid treatment (table V, fig 5)

Discussion

The main new result in this study is the reduction of the TG pools of heart and red muscle in response to nicotinic acid It is, for the following reasons possible that this effect is due to the inhibition of FFA mobilization caused by nicotinic acid In the fasting state, as used in these rats FFA contributes to more than 50 per cent of the expired carbon di

TABLE V Values for cholesterol phospholipids and triglycerides in the red muscle of the gastrocnemius muscle of fasted rats injected with saline (C) or nicotinic acid (N)

Time after injection		2 hours			4 hours			6 hours		
		C	N	P	C	N	P	C	N	P
Cholesterol	M	1 6	1 6		1 7	1 6		1 8	1 9	
(mg/g)	SEM	0 1	0 0	>0 05	0 1	0 1	>0 05	0 1	0 1	>0 05
	n	10	10		11	11		11	11	
Phospholipids	M	18 1	18 0		17 8	17 6		17 1	17 3	
(mg/g)	SEM	0 5	0 5	>0 05	0 5	0 4	>0 05	0 5	0 4	>0 05
	n	10	10		11	11		11	11	
Triglycerides	M	4 09	2 61		3 48	1 55		3 12	1 79	
(μmole/g)	SEM	0 41	0 40	<0 05	0 27	0 16	<0 001	0 31	0 23	<0 001
	n	10	10		11	11		11	11	

Symbols as in table I

oxide *It is well established from in vivo* and in vitro data that FFA are taken up and oxidized by the myocardium as well as by the skeletal muscle FFA taken up by muscle tissues are incorporated into TG and phospholipids in the tissues The detailed pathways during oxidation of fatty acids by muscle tissues are not known It is known, however, that when muscle slices are incubated in vitro in substrate free media they continue to respire with a RQ indicating oxidation of lipids (21) and that the lipid content decreases (18) There is also good evidence that the isolated perfused heart can respire without exogenous substrate by utilizing endogenous lipids (19) The situation induced by nicotinic acid may be similar to incubation at a low substrate level as the turnover of FFA in plasma is reduced Furthermore the total oxygen consumption is not reduced by nicotinic acid in man (6 11, 15) The present metabolic situation may thus be characterized by reduced availability of FFA to the muscles but an unchanged rate of oxidation, which as judged from values of RQ (6, 11, 15) may be furnished by fatty acids The decrease of the TG pools in tissues such as the heart and skeletal muscle demonstrated here as the result of nicotinic acid suggests that these TG pools deliver fatty acids to the mitochondria for oxidation The finding that the phospholipid content did not decrease suggests that the TG pools are the main storage form of fatty acids for immediate oxidation in heart and red muscle The results also indicate that nicotinic acid does not block TG lipolysis in muscle tissues

In white muscle on the other hand no significant changes occurred in the TG content Available evidence suggests that this type of muscle has a metabolism which is different from the red muscle White muscle has in comparison to red a smaller amount of oxidative enzymes a lesser rate of lipid oxidation and a

greater rate of anaerobic glycolysis (1, 13, 14, 17) This type of muscle may thus depend more on glycolysis than on lipid oxidation for its energy needs In this context it is of interest that in the condition opposite to inhibition of FFA mobilization, i e during excessive FFA mobilization, the red muscle, heart muscle and liver significantly increase their content of histologically stainable lipids, while white muscle does not (7)

The disappearance of TG pools during reduced FFA mobilization and the increase in lipid pools in heart and skeletal muscle in conditions where FFA mobilization exceeds the need of substrate for oxidation strongly suggests that these pools act as reservoirs for substrate During periods of enhanced availability of circulating FFA they are replenished and are thus available as substrate when circulating FFA is low This mechanism may neatly balance over time the caloric homeostasis

Summary

Fasted rats were given saline or nicotinic acid subcutaneously and the lipid content of plasma, liver, heart and skeletal muscle followed at 2, 4 and 6 hours after the injection

Nicotinic acid treatment caused the following changes

Plasma FFA levels reduced up to 4 hours, triglycerides and phospholipids depressed up to 6 hours and cholesterol values lowered at 4 and 6 hours

Liver Triglyceride values reduced at 2 and 4 hours and phospholipid values up to 6 hours No changes in cholesterol content

Heart Significant depression of triglycerides at 2 hours and slight elevation of phospholipids at 4 hours, otherwise no changes

Skeletal muscle No significant effect on lipids in white muscle fibres Depression of the triglyceride content in red fibres up to 6 hours but no changes in amount of cholesterol and phospholipids in this fibre type

The reduction of triglyceride pools in various tissues after inhibition of FFA mobilization by nicotinic acid may reflect the consumption of these pools for oxidative purposes in a situation characterized by low availability of circulating substrate It is suggested that local tissue lipid pools serve an important function as reservoirs in the calorigenic homeostasis, balancing the rate of FFA mobilization and the need of fatty acids for oxidation

Acknowledgement

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From the Department of Neurology (Head S Refsum M D) University Hospital Rikshospitalet Oslo and the Department of Physiology (Head W Velle V M D and Former Head H Stormorken M D V M D), Veterinary College of Norway, Oslo Norway

Histamine Formation after Oral L-histidine in Health and Dystrophia Myotonica

By

Ottar Sjaastad

Oral loading with L-histidine in healthy human subjects (7) dogs (7) and guinea pigs (19) results in a transient augmentation in the urinary excretion of free histamine Irvine et al (7) conclude that this extra urinary histamine in the dog as well as in man is formed by intestinal micro-organisms since no increase was observed after pretreatment with oral antibiotics

L-histidine is, however, actively absorbed from the jejunum and ileum (12, 21), and the flora of the small intestine is sparse (2, 8) Although it is known that intestinal micro-organisms can produce histamine in vitro (5), this production seems to be moderate even in the feces (O Sjaastad, unpublished data) These findings cast some doubt on the observations of Irvine et al (7) This problem has therefore been reinvestigated

We have also attempted to clarify another problem Patients with dystrophia myotonica (15) excrete increased quantities of N acetylhistamine in the urine A close correlation exists between the degree of acetylhistaminuria and the fecal concentration of histamine like activity (HA) in these patients The most plausible explanation of these findings (15) is increased intestinal formation of HA and/or a defect in the degradation of formed HA

L histidine is known to be the precursor of histamine Since ingestion of identical food did not alter the divergent pattern (15) ingested histidine could possibly have a different fate in patients and controls A study of the response to oral L histidine loading in these patients therefore seemed indicated

Material and methods

After a control period of 1—3 days 10 g L-histidine monohydrochloride monohydrate (Sigma) was administered orally at 8 a m after overnight fasting In some cases L-histidine was also given on the subsequent morning Eight studies were performed in 6

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healthy individuals, whereas 7 patients with dystrophia myotonica were studied

A mixed diet was ordinarily given during the study A standard diet, however, was given 3 days prior to, the day of, and following the L-histidine loading in the studies in table I This diet consisted of 2 slices of bread with cheese and 400 ml milk at set times 3 times a day Furthermore a solution of 5 g L-histidine in normal saline was injected into the ascending colon of 2 patients undergoing gynecological operations These patients had no history of gastrointestinal trouble Details regarding anesthesia collection of urine etc in these patients are described elsewhere (18)

L-histidine 10 g dissolved in distilled water was administered rectally to one healthy individual To the same individual L-histidine 2 g per day for 6 days was administered rectally just after defecation Urine was collected for 24 hours after administration

Urinary histamine was estimated as described in detail elsewhere (3 16)

The unweighted mean and range for free histamine in urine in the control series were 12 6 _ 6 3 (SD) and 2 0 — 31 0 μg base/24 hours (16) The corresponding values for conjugated histamine were 30 0 ± 23 6 and 1—99 The highest single observation for conjugated histamine was 130 μg/24 hours

Fecal HA was estimated as described elsewhere 17) In the control series (17) fecal HA was always 0 17 μg base/g wet weight

The values for histamine in this study are expressed in terms of the base and represent the mean of duplicate analyses They are uncorrected for losses during the extraction procedure

Results

To the subjects in table I L histidine was administered once Table II summarizes the results from studies in which a mixed diet was given, and in which L histidine was administered on two consecutive days

Free histamine in urine

Increase in urinary excretion of free histamine invariably followed oral L histidine in both controls and patients (null hypothesis P < 0 05 for both) When the excretion on the last control day was compared with that on the day of loading, the mean increment in healthy individuals was 31 μg/24 hours There was a smaller increase in urinary free histamine in patients with dystrophia myotonica (average increment 1 88 times) than in the controls (average increment 2 71 times) This difference was not significant (0 1 > P > 0 05) Diamine oxidase (Sigma) inactivated almost completely the biological activity appearing in urine after loading

Conjugated histamine in urine

In controls (tables I and II) no significant difference existed between the last control excretion of conjugated histamine and the highest postloading excretion (P > 0 2, null hypothesis) In patients with dystrophia myotonica, a significant increment occurred by the aforementioned criteria (P < 0 05)

Increase in urinary output of conjugated histamine took place both in patients with control excretions within the normal limits, and in patients with increased control excretions An increase however, was not found invariably In the study group a significant difference did not exist between the last control excretion and the excretion on the day of L histidine loading

TABLE I Urinary and fecal content of histamine after oral L-histidine

	Control				Histidine loading	Post loading		
	Day no	1	2	3	4	5	6	7
Healthy individuals								
T	Free		10	16	25	14		
	Conj		17	2	1 2	28		
	HA		<0 2			<0 19 0 07	<0 04	
O	Free		12		22	14	30	
	Conj		5	[1]13	14	1 3	12	
	HA		<0 04				<0 05	
S	Free	12	18	17	29	13	14	
	Conj	51	28	34	14	30	23	
	HA	<0 1		<0 2	<0 14	<0 07		
H	Free	14	19	18	40	14	14	
	Conj	100	210	240	250	190	390	
	HA	0 2		7 6		0 1	4 2	
Dystrophia myotonica								
2	Free	59	48	71	160	79	170	
	Conj	1 100	4 300	6 300	35 000	17 000		
	HA		23	140	850	130	130	120
15	Free	4	3	3	5	9	5	
	Conj	52	58	70	64	130	240	
	HA		0 2	0 24	0 21	13		0 2
23	Free			81	110	70		
	Conj			580	360	450		
	HA	0 7	0 53	0 9	11	19	11	

A standard diet was ingested during this study
Free and *Conj* refer to urinary excretion of free and conjugated histamine in urine in $\mu g/24$ hours.
HA means histamine like activity in the feces in $\mu g/g$ wet weight
[1] Total (free + conjugated) histamine

Histamine in the feces

In the controls no clear elevation of fecal HA appeared after L-histidine loading although borderline high fecal HA (i e 0 1—0 15 $\mu g/g$ wet weight) was observed at times Control H (table I) seemed to be an exception He showed increased preloading levels of both urinary conjugated histamine and fecal HA

A significant difference existed between the last control value and the highest post loading value in patients with dystrophia myotonica ($P < 0\ 05$ null

TABLE II Urinary and fecal excretion of histamine after oral administration of L-histidine monohydrochloride monohydrate 10 g on 2 consecutive days

		Control			Histidine loading		Post loading	
		Day no	1	2	3	4	5	6
Healthy individuals	B	Free		10	39	43	15	18
		Conj		120	61	35	27	39
		HA			0 15			
	K	Free		57	130	100	90	
		Conj		43	68	33	55	
	T	Free	7	13	53	44		
		Conj	7	16	0	5		
		HA			0 07	<0 09		
	O	Free		14	45	36		
		Conj		20	18	29		
		HA					0 1	
Dystrophia myotonica	[1]13	Free	13	15	21	25		
		Conj	28	11	85	96		
		HA	1 1		1 4	9 3		
	7	Free		10	16	22		
		Conj		290	220	150		
		HA		7 8		11		
	14	Free		17	43	18		
		Conj		36	15	80		
		HA			0 43	0 44		
	20	Free		24	57	56		
		Conj		50	83	130		

Free and *Conj* refer to urinary excretion of free and conjugated histamine in $\mu g/24$ hours
HA means histamine like activity in the feces in $\mu g/g$ wet weight
[1] Control excretions were measured a few days after oral phthalylsulfathiazole treatment

hypothesis) In this calculation the fecal HA of patient No 23 (table I) on the loading day (i e 11 μg b) was considered to be a preloading value since the pertinent defecation took place only 7 hours after the loading This fecal concentration thus probably had connection with the preloading urinary output of conjugated histamine, viz 580 $\mu g/24$ hours

L histidine loading for 3—4 days

In two different experiments, one healthy individual (O, table III) was given 10 g L histidine for 3 and 4 consecutive days In the first experiment no augmentation in urinary output of conjugated histamine was observed A fecal HA of 0 1 $\mu g/g$ wet weight was found on the third day of loading In the last experiment the urinary conjugated his

TABLE III Urinary and fecal excretion of histamine after administration of L-histidine rectally or into the colon in controls

		Control excretion	Hours after loading 0—6 h	6—24 h	0—24 h	Mode of L-histidine administration
E	Free	3	5	10	15	Injection at
	Conj	15	20	67	87	Bauhin s valve (5 g)
A	Free	4			Traces	Injection in
	Conj	36			60	ascending colon (5 g)
O_I	Free	16 (9—30)[1]	[2] 4	12	16	Rectal instillation
	Conj	15 (1—45)[1]	[2]10	100	110	(10 g)
			Day of loading 4		6	
O_{II}	Free	16 (9—30)[1]	4		5	
	Conj	15 (1—45)[1]	110		75	Rectal instillation
	Fecal histamine		[3]0 13/0 21		[3]0 58/2 4	2 g daily for 6 days

Free and *Conj* refer to free and conjugated histamine in the urine ($\mu g/24$ hours)

[1] Mean and range of excretion

[2] Collection of urine from 0—4 1/2 h as defecation took place at 4 1/2 hours

[3] The figures mean histamine like activity and conjugated histamine ($\mu g/g$ wet weight)

tamine on the third day was still 20 $\mu g/24$ hours The fecal HA however was elevated on the third day it was 0 88 and on the fourth day 0 62 $\mu g/g$

Administration of L histidine to the large bowel

Table III summarizes the results from experiments with administration of L-histidine rectally or directly into the colon of controls

L-histidine loading significantly increased the urinary conjugated histamine in these experiments ($P < 0\ 05$ No definite increment in urinary free histamine appeared In experiment O_I (table III) such severe colicky abdominal pains appeared that defecation took place after 4 1/2 hours

Discussion

In vitro formation of histamine from L histidine has been demonstrated in various human tissues (1) In man however no conclusive evidence exists of histamine formation in vivo (1)

In the present study a significant rise in biological activity in the urine without a concurrent rise in urinary conjugated histamine was found in controls after oral L histidine loading The administration of 200 mg histamine diphosphate to

various segments of the gastro intestinal tract gives an entirely different pattern a small, but not significant, increment in urinary output of free histamine is noted, whereas the increment in urinary excretion of conjugated histamine is invariably marked (18)

These findings strongly indicate that the excess urinary biological activity after an oral load of L-histidine is not of intestinal origin The low fecal HA gives additional support to this view It is conceivable that the fate of histamine possibly originating intraluminally could be entirely different from that of histamine injected intraluminally The results of the experiments with administration of L histidine to the large bowel of controls, however, suggest that when histamine is formed intraluminally, a rise in urinary conjugated histamine results

The question also arises whether bioactive substances different from histamine could explain the rise in urinary biological activity The fact that the activity was antagonized by antihistamine and almost completely inactivated by diamine oxidase makes this assumption unlikely The bioactive side chain N methylhistamines are, e g, not inactivated by histaminase (9)

Urines from these subjects were not cultured Formation of all the excess histamine in the urine is highly unlikely, since most subjects were in good health The possibility that L histidine or its metabolites have occasioned histamine release in the tissues or altered the catabolism of histamine seems somewhat remote

The reason why Duner and Pernow (4) did not find any increase in urinary free histamine after oral L-histidine is probably that too small a dosage was used, i e 5 g Due to the pronounced intraindividual variation in urinary output of free histamine (16) minor increments following 5 g of L histidine may be swamped in 24 hour samples

After completion of the present experiments, the author became aware of a study by Oates et al (13), who used a fluorometric technique for the estimation of urinary histamine After oral L-histidine loading these investigators found increments in urinary free histamine of equal magnitude before and after oral antibiotics Their study thus indicates that the increment in urinary free histamine after oral L histidine is not caused by intestinal bacteria The present study seems to have a wider inference than that of Oates et al the excess urinary histamine is probably not formed intraluminally

It should, however, be mentioned that the findings of Oates et al have been questioned by Waton (20), who thinks that they may be due to the method used Beall (1) thinks that in Oates et al 's study inadequate doses of antibiotics might explain their lack of effect

The kidneys are rich in L-histidine decarboxylase and have been suggested as a site of histamine formation after L histidine loading (6, 13) In this connection mention must be made of the fact that no untoward effects whatever were noted by the healthy individuals after loading

10 g of L-histidine monohydrochloride monohydrate corresponds to approximately 7 4 g of the free amino acid After intravenous and subcutaneous injec

tion of C[14] histamine, 1—5 % is excreted in the free form in the urine (10, 14) The fraction excreted as free histamine would thus average 3 % The mean recovery of histamine added to urine was 72 % in our experiments (16) On the assumption that intrinsic histamine is not metabolized entirely differently from parenterally administered histamine, it is then possible to calculate approximately the histamine formed in the tissues as a percentage of administered L histidine, i e 0 02 %

The statement by Oates et al (13) that synthesis of histamine by intestinal flora does not contribute appreciably to unconjugated urinary histamine under normal circumstances' does not seem to be entirely supported by their experimental evidence Ingestion of L-histidine in pure form seems to differ materially from ingestion of L histidine as a component of food especially poorly digested food Ingestion of such food may leave a larger proportion of L-histidine unabsorbed and available for bacterial degradation Along the same lines, ingestion of meat is occasionally associated with increased urinary output of conjugated histamine (11) The possibility of intestinal histamine production is especially interesting in view of the finding that high spontaneously occurring fecal HA is occasionally associated with moderately increased urinary levels of free histamine (15)

The possibly unabsorbed fraction of L-histidine could not be approximated in the experiments with oral L histidine loading Therefore the amino acid was administered to the large bowel There was indirect evidence for limited formation of histamine intraluminally in these experiments, but not of the magnitude as to be associated with an appreciable increase in urinary free histamine

More pronounced formation of histamine in the large bowel could conceivably take place if the microflora were exposed to an environment with increased concentration of L-histidine for longer periods of time Administration of L-histidine via the anal route daily for 6 days did not lend support to this hypothesis The experimental conditions however, were not ideal Thus there is so far no direct evidence for marked intraluminal formation of histamine in healthy human individuals The possibility of course remains that histamine is formed to a considerable degree in the intestine but is degraded so quickly that it cannot be detected in urine or feces

A striking discrepancy in the response to oral L-histidine loading however, existed between healthy individuals and patients with dystrophia myotonica In these patients the fecal HA and urinary conjugated histamine increased significantly whereas the urinary histamine increased somewhat less than in healthy individuals This pattern is taken to indicate that a small fraction of L histidine has been converted to histamine intraluminally

Summary

Oral L-histidine loading (10 g L histidine monohydrochloride monohydrate) was performed in 6 healthy individuals and 7 patients with dystrophia myotonica

1 In healthy individuals administration of L-histidine by mouth resulted

in a significant increase in urinary free histamine in 24 hour samples without concomitant rise in urinary conjugated histamine or fecal histamine like activity This strongly suggests that the extra urinary histamine is formed in the tissues and not in the intestines

2 In patients with dystrophia myo tonica the response was different a significant augmentation in urinary free histamine was associated with increased levels of urinary conjugated histamine and increased concentration of fecal histamine like activity This pattern is taken to indicate that these patients after oral L histidine loading form his tamine both inside and outside the lumen of the intestines

Acknowledgements

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From the Cardiovascular Laboratory of Medical Department B (Head A Tybjærg Hansen) Rigshospitalet (University Hospital) Copenhagen Denmark

The Late Systolic Murmur Recorded in the Coronary Sinus with Intracardiac Phonocardiography

By

ALF WENNEVOLD

The late systolic murmur has for many years been considered to be an extra cardiac variant of functional, innocent murmurs But recent evidence based on left ventricular cineangiocardiography points to mitral regurgitation as the cause of a late systolic murmur (1, 4, 6)

The diagnosis of mitral regurgitation was recently made in one case mainly by recording a late systolic murmur in the left atrium by intracardiac phonocardiography (3)

In this paper it is reported, that the late systolic murmur in two patients was recorded with a phonocatheter in the coronary sinus during a simple right heart catheterization thereby giving supporting evidence to the mitral origin of the murmur

Case reports

Case no 1 A 40 year old woman was admitted for evaluation of possible heart disease She gave no history of rheumatic fever Ten months previously she was admitted to another hospital because of sudden left sided chest pain which occurred while she was working in the garden The pain subsided after half an hour and no cause for it was found The day before she had received an unusual hearty hug from her husband She had been asymptomatic since

Physical examination The blood pressure was 110/60 mm Hg and the heart rate was 68—72 per min The arterial pulse was normal The point of maximal impulse was in the fifth intercostal space in the midclavicular line At the apex a rough medium to high pitched grade 2 (of 6) late systolic murmur was heard (fig 1) the murmur radiated with slightly decreasing intensity to the left axilla and to the posterior left chest but was not heard at the base No diastolic murmur was heard The second heart sound over the pulmonary area was normally split during inspiration and single during expiration A third heart sound was present at the apex (fig 1) The electrocardiogram and the chest roentgenogram were normal

Case no 2 A 25 year old woman was admitted for evaluation of a heart murmur She had for the last 10 years been treated for anorexia nervosa There was no history of rheumatic fever and her only cardiac

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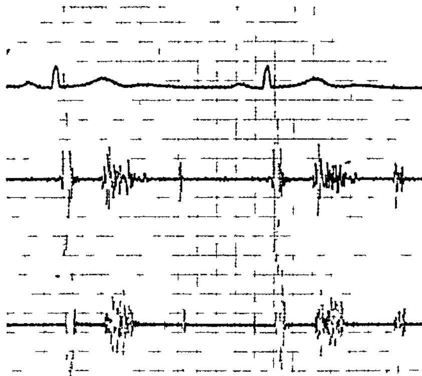


Fig 1 External phonocardiogram from the apex of case no 1 showing late systolic murmur (mingograf 31 B Elema Schonander recording in the 100 cps range (middle tracing) and in the 400 cps range (lower tracing))

symptoms were shortlasting paroxysms of tachycardia

Physical examination She was 169 cm tall and weighed only 37 7 kg The blood pressure was 120/70 mm Hg and the heart rate 100 per min The arterial pulse was normal The point of maximal impulse was in the fourth intercostal space just within the midclavicular line At the apex a blowing medium to high pitched late systolic murmur was heard (fig 2) the murmur was preceded by a click The murmur was grade 3 (of 6) and radiated with decreasing intensity to the left axilla and to the posterior left chest but was very faint over the base No other murmur was heard The second heart sound over the pulmonary area was abnormally split (0 06 seconds in held expiration) with normal variation of the splitting during respiration No third heart sound was heard The chest roentgenogram was normal, the electrocardiogram showed inverted T waves in lead II and III and flat T waves in V_{5-6} but was otherwise normal

Right heart catheterization was performed with the Allard Laurens micromanometer (5) in both patients (table I) The presence of left to-right shunt was excluded with determination of oxygen saturation and with the platinum electrode and hydrogen inhalation

Intracardiac phonocardiography In both patients a late systolic murmur was recorded in the coronary sinus (figs 3 and 4 The murmur was identical in time to the externally heard murmur and disappeared when the catheter was withdrawn to the right atrium The position of the catheter tip in the coronary sinus was verified by the low

oxygen saturat on No other murmur was recorded in the r ght s de of the heart

A d agnosis of m ral insuffic ency was made in both pat ents The history of chest trauma and ep sode of pain n case no 1 was thought poss bly to account for damage of a pap llary muscle or the chordae tendineae with result ng sl ght mitral regurgitat on caus ng the late systol c murmur No clue as to the aet ology was found n case no 2



F g 2 Ex ernal phonocard ogram f om the apex of case no 2 show ng the la e sys ol c murmu preceded by a cl ck

Discussion

It has been documented that the holosystol c murmur of mitral insuff c ency may often be transm tted to the coronary sinus (2) This has been expla ned by the prox m ty of the coronary s nus to the poster or m tral comm sure The presence in the coronary s nus of a systol c murmur of any other or g n has h therto not been reported and in this laboratory has only been found in a few patients w th ventricular septal de fect Th s condition was eas ly ruled out n the present t vo cases

As the orig n of a late systol c murmur seems to be m tral insufficiency (1 4 6) it should be poss ble to record th s murmur as well as the holosystol c murmur in the coronary s nus Supporting evi dence of a cl n cal d agnosis of m tral insuff ciency may thus be obtained by means of intracard ac phonocard og

TAB E I Results of r gh hea t ca he er za on

Case no	P essures a rest				Pressu es dur ng exercise		Card a ndex	
	Right atr um (mm Hg)	R ght en r cle (mm Hg	Ma n p a mm H	Wedge p a mm Hg)	Main p a (mm Hg	Wedge p.a mm Hg	At es l m	During exer ise l m
1	2	18 2	17 7	7	25 10	10	2 4	4 0
2	2	23 2	20 6	5			3 2	

p a pu monary ar ery

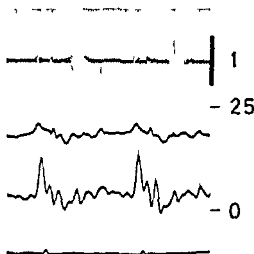


Fig 3 Intracardiac phonocardiogram (upper tracing) from the coronary sinus in case no 1 showing late systolic murmur In the upper right margin a calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line Pressure is recorded both with the micromanometer at the tip of the catheter (middle tracing) synchronously with the sound and through the sidehole (lower tracing) 1 5 cm from the tip the latter tracing being calibrated to 0 and 25 mm Hg

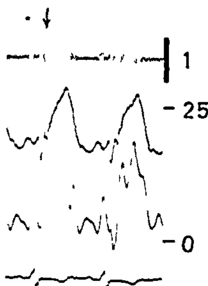


Fig 4 Intracardiac phonocardiogram from the coronary sinus in case no 2 The arrow marks the click.

raphy in the right side of the heart, as it was done in the present cases, though the diagnosis was not proved

Summary

The first two cases are reported, in which a late systolic murmur was recorded in the coronary sinus with intracardiac phonocardiography, thereby suggesting mitral insufficiency as the cause of the murmur

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From the Medical Clinic B (Renal Clinic) and the Department of Pathology University of Lund Lund Sweden

Experimental Studies on Charcoal Haemoperfusion in Phenobarbital Intoxication and Uraemia, Including Histopathologic Findings

By

KARL ERIK HAGSTAM LARS ERIK LARSSON and HANS THYSELL

Extracorporeal haemoperfusion through activated charcoal has since 1964 been applied on a small scale both in experiments on animals and in man (3 6, 7 8) The method has been presented as an alternative or a complement to dialysis treatment in endogenous and exogenous intoxications for instance uraemia and barbiturate poisoning

Results of histological examination of internal organs from human subjects or animals treated by haemoperfusion through charcoal have not hitherto been published

We present here a modified technique for perfusion of the blood through activated charcoal suitable for use in experiments on rabbits The perfusions were made on untreated control animals and on phenobarbital intoxicated and on uraemic animals The rabbits were killed at varying times after the perfusions and their internal organs were examined histologically The results in the phenobarbital series were compared with Alwall s et al (2) results of treatment of phenobarbital poisoning in rabbits by forced polyuria, exchange ultrafiltration and haemodialysis

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Survey of the literature

Yatzidis (6 7) reported laboratory experiences with a charcoal haemoperfusion apparatus It consisted of a siliconized glass cylinder filled with 200 g of activated charcoal in granulate form and of particle size 0 50—0 75 mm (Merck) Stainless steel filters with 0 15—0 20 mm meshes were mounted at both ends The cylinder was well washed with deionized tap water and sterilized by heating to 120 C for 2 hours Sterile physiological saline solution was then run through the column and the apparatus was finally primed with about 200 ml of the same solution containing 100 mg heparin per litre

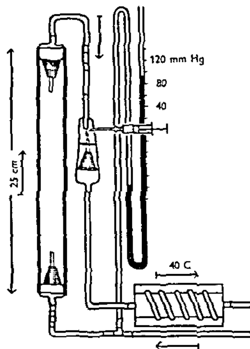


Fig 1 Apparatus for charcoal haemoperfusion in rabbits

With this apparatus Yatzidis performed haemoperfusion on healthy dogs and on patients with terminal chronic renal failure Pyrogenic reactions and a transient drop in blood pressure were noted in some cases No destructive effects on the formed elements of the blood were seen, except for thrombocytopenia in a few cases Creatinine, uric acid indican phenolic compounds, guanidine bases and organic acids were effectively removed from the blood The adsorption of urea, magnesium, and phosphates in serum was poor Other serum eletrolytes did not change significantly A low pH of the blood was corrected

Dunea and Kolff (3) applied a similar technique for haemoperfusion in healthy dogs and in 3 patients with chronic renal failure The perfusion equipment was sterilized by gas In their experiments they found a significant reduction of the serum calcium concentration and of the thrombocyte count In other respects Yatzidis's observations were verified

In 1965 Yatzidis et al (8) reported the successful application of the haemoperfusion through charcoal in the treatment of 2 cases of severe barbiturate poisoning On becoming conscious the patients experienced transitory symptoms, such as a general feeling of unrest, shortness of breath, facial flushing, and a burning sensation in the throat, urethra, and anus The authors believed that these symptoms were attributable to sulphur compounds liberated from the charcoal

Methods

Apparatus (fig 1)

A plastic tube 25 cm in length and with a 19 mm bore, was carefully filled with about 23 g of activated charcoal in granulate form and of particle size 0 3—0 5 mm (Merck) in 5 cases 0 5—0 75 mm (Merck) in 15 cases and 0 5—1 0 mm (Grave) in the rest of the cases An infusion set filter of nylon with 0 180 mm mesh (Wipex AB) was mounted at each end of the tube Silastic tubes were connected to both ends of the column and the apparatus was sterilized by boiling in deionized distilled water for 30 min The column was then rinsed with 1/2 l of boiling deionized distilled water and 2 l of sterile isotonic glucose saline solution (5 4 g NaCl and 22 g glucose per l) The rinsing was done intermittently with varying positive and negative pressures In the later part of this procedure the rinsing fluid was free from macroscopically observable charcoal particles Centrifugation of the last rinsing fluid revealed however continual occurrence of microscopic charcoal particles

The column of charcoal was placed vertically The blood was channeled to pass from below upwards so as to avoid jamming with charcoal granulates at the bottom of the column A mercury manometer was connected with the arterial end for blood pressure and perfusion pressure measurements

A drip chamber (Wipex AB) with a filter as described above was attached to the venous side of the column It served as a trap for air and blood clots and as a flow meter A fine needle inserted in the upper part of the drip chamber permitted control of the fluid level in the chamber The outlet silastic tubing passed through a water bath at 40 C The perfusion system which held about 40 ml was filled with physiological saline and 30 ml of this solution were then replaced by citrated rabbit blood to which 10 mg of heparin had been added The pressure fall in the column packed with charcoal granulate of particle size 0 5—0 75 mm at a flow rate of 10 ml per minute was about 40 mm Hg The resistance increased with decreasing particle size

Technique of operation

The animals were fixed in the supine position The carotid artery and the jugular vein on one side were dissected free under local anaesthesia with 1 % Xylocaine solution After haemostasis 15 mg of heparin were given intravenously Glass cannulae were inserted into the vessels (1) Titanium electrodes for recording of EEGs were introduced surgically under Narkotal anaesthesia in 7 animals 2 to 4 weeks before the perfusion Two electrodes were placed extradurally and fixed to the scalp over the anterior part of the parietal cortex on the left and right sides (5)

Perfusion

The glass cannulae were connected with the extracorporeal system Blood pressure pulse rate ECG (standard lead II) respiratory rate rectal temperature perfusion pressure and flow rate were recorded at 15 minute intervals EEG recordings (a bipolar lead between the inserted electrodes) were made in 7 cases A flow rate exceeding 3 ml per minute was regarded as good a flow rate of 2—3 ml per minute as moderate and below 2 ml per minute as poor

A continual sparse intermixture of charcoal particles of the order of 5—35 μ was noted in smears of blood taken on the venous side between the drip chamber and the cannula Repeated rinsing of the columns with up to 10 l of glucose saline and perfusion with 10 % Macrodex solution or blood did not prevent the intermixture of charcoal

By means of simple couplers the column of charcoal could be rapidly replaced by another The new column was almost filled with blood from the old one Up to four column changes per treatment were made without technical complications and with insignificant losses of blood At the end of the perfusion the artery was ligated and 30 ml of the blood in the perfusion system were then transfused into the rabbit The vein was ligated and the skin sutured Before the perfusion 0 15 ml of Streptopenin (Kabi) were given intramuscularly for prophylactic purposes (30 000 i u of penicillin procaine plus 0 04 g of streptomycin)

Experimental results

Control series (11 rabbits)

Eleven untreated rabbits (weight range 2 900—3,810 g) were treated by haemoperfusion through activated charcoal for 30—210 minutes One to three columns were used The flow rate was good in all the cases The general condition of the animals did not change during the treatment Slightly lowered muscular tension was noted however in the first post perfusion hour which may be attributable to the fixation in the supine position Blood pressure pulse rate electrocardiographic pattern respiratory rate and rectal temperature

TABLE 1 Some laboratory findings before and after haemoperfusion through charcoal in control rabbits and uraemic rabbits

Serum	Control series			Uraemia series		
	No of cases	Perfusion Before	Perfusion After	No of cases	Perfusion Before	Perfusion After
Sodium (mEq/l)	5	142	142	4	128	129
Potassium (mEq/l)	5	5 3	5 1	8	9 6	9 8
Calcium (mEq/l)	3	7 3	6 5	3	7 4	6 4
Phosphate (mg/100 ml)	3	4 4	3 8	3	3 7	3 3
Creatinine (mg/100 ml)	5	1 02	0 55	8	8 1	4 2
Uric acid (mg 100 ml)	3	0 5	0 1	4	1 1	0 3

showed no significant changes during he perfusion Blood analyses were nade before and after the perfusion in 5 ases The results of these analyses greed with previously published data 3, 6) in that the findings were as ollows A significant fall in the serum evels of uric acid and creatinine Blood N P N serum calcium and serum phosphate fell slightly Serum sodium and serum potassium did not change (table 1) Serum electrophoresis and serum levels of cholesterol and bilirubin studied in 2 cases did not change

The animals were killed by exsanguination one immediately after the perfusion and the others after varying intervals of time 2 days to 9 months after perfusion The animals behaved normally during the observation period and exhibited no signs or symptoms of disease The autopsies showed no macroscopical abnormalities The microscopical findings will be reported in a later section of this paper

Series of phenobarbital intoxicated animals (13 rabbits)

The series comprised 13 rabbits (weight range 1,900—3,000 g) The experimental conditions resembled closely those used by Alwall et al (2) in their study of the effect of polyuria exchange ultrafiltration, and haemodialysis in phenobarbital intoxicated rabbits Accordingly 130 mg of phenobarbital per kg body weight were given by deep intramuscular injection The animals were comatose after 4 6 hours Perfusion through charcoal was then carried out for 20 195 minutes by the technique described above One to four charcoal columns were used 50 ml of glucose saline per kg body weight were given intravenously

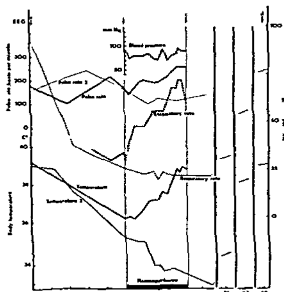


Fig 2 Some clinical data from two phenobarbital intoxicated rabbits no 1 treated with haemoperfusion through charcoal no 2 untreated (see text) The arrows refer to the EEG recordings in fig 3

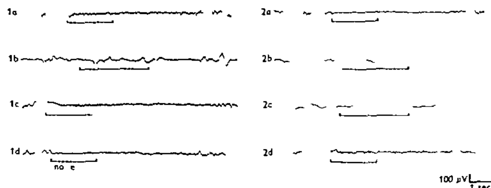


Fig 3 Bipolar EEG recordings from left and right parietal regions from the two phenobarbital intoxicated rabbits presented in fig 2

on the day of perfusion and on the next day until the animals woke up One intoxicated animal was left untreated but received fluid and prophylactic antibiotics as described in the foregoing Data from this experiment are set out in figs 2 and 3 for comparison with data from a treated animal For comparison with a control series the reader is referred to the publication of Alwall et al (2)

The depth of anaesthesia decreased during the course of treatment in all the perfused animals The respiratory rate blood pressure and rectal temperature rose fig 2 The EEGs before the perfusion were grossly pathological and showed no reaction to sound or pain

TABLE II *Some technical data from the perfusions and the duration of narcosis in 13 phenobarbital intoxicated rabbits treated by charcoal haemoperfusion*

Rabbit no	Flow rate (ml/min)	No of columns	Perfusion time (min)	Size of charcoal particles (mm)	Duration of narcosis (hours)
1	>3	4	195	0 5—0 75	12 3
2	>3	3	135	0 5—0 75	12 5
3	>3	2	145	0 5—1 0	9 3
4	>3	2	120	0 5—0 75	7 8
5	>3	2	120	0 3—0 5	12 5
6	>3	1	60	0 5—1 0	10 5
7	>3	1	20	0 5—1 0	29 8
8	2—3	2	90	0 5—1 0	29 0
9	2—3	1	180	0.5—1 0	25 3
10	2—3	1	125	0 3—0 5	16 3
11	<2	1	150	0 5—0 75	26 3
12	<2	1	90	0 5—1 0	14 3
13	<2	1	20	0 5—1 0	30 8

stimuli During the perfusion there were gradual regression of the abnormal pattern and return of the reaction to sensory stimuli (fig 3) The increase of wakefulness was to some degree correlated with the flow rate and the number of charcoal columns used (table II) The flow rate was good in 7 rabbits and they were able to sit steady after 13 5 (7 8—29 8) hours In 3 cases of moderate flow rate the animals could sit steady after 13 3, 25 3 and 29 0 hours respectively Three animals in which the flow rate was poor could sit steady after 14 3 26 3 and 30 8 hours respectively Analyses of blood serum and urine were not made After waking the animals behaved normally and showed no signs or symptoms of disease They were killed by exsanguination on the 4th to 78th post perfusion day Autopsies showed no macroscopical abnormalities The microscopical findings will be reported later in this paper

Series of uraemic animals (6 rabbits)

In 6 rabbits (weight range 2,190—2,910 g) the ureters on both sides were ligated 2 or 3 days before the planned perfusion The animals were perfused for 40—120 minutes, one or two columns were used The flow rate was good in 5 cases and moderate in 1 case The rabbits were sluggish before the perfusion and the condition was unchanged after the perfusion Blood pressure pulse rate electrocardiographic pattern respiratory rate, and rectal temperature did not change There were significant falls in the serum levels of uric acid and creatinine and slight falls in blood NPN and serum phosphate Sodium and potassium concentrations in the serum did not change (table I The

animals died on the 4th — 5th post-perfusion day Thus, the survival time was slightly longer than ordinarily (4) Autopsies showed no macroscopical abnormalities except for a few dark-brown patches, up to 4 mm in diameter, on the lung surfaces in 2 cases The microscopical findings will be reported below

Microscopical examination

Forty rabbits were examined histologically Six of them had not undergone perfusion through charcoal In none of these six were any charcoal particles found in the lungs liver, spleen, or kidneys All the 34 perfused animals had charcoal particles in their lungs In 28 of them charcoal particles were also found in the spleen, in all the cases being less numerous and of smaller size than those in the lungs In 14 animals charcoal particles were demonstrated in the liver, and in all of these, except one in the spleen as well In 9 animals sparse amounts of charcoal were found in the kidneys In 2 cases the presence of a few small charcoal particles in the brain was suspected but could not be established No charcoal in the heart was seen in any of the cases

The histologically observable amounts of charcoal in internal organs proved to be correlated with the rate and the duration of perfusion and the number of columns used but not with the kind of charcoal used

The charcoal in the lungs was present both as small particles wedged in the capillaries of the alveolar walls and as larger conglomerations in small arteries



Fig 4 Lung section with charcoal particles in small arteries and capillary vessels Section 15 μ thick Kernechtrot × 90

(figs 4 5 and 6) The tissue reaction around the particles was insignificant, being seen only as slight endothelial proliferation, in some cases with formation of a few histiocytic giant cells There was no fibrosis In the spleen the charcoal was present as small particles mostly distributed within the red pulp In the liver it occurred as small particles in the sinusoids and in 1 case profusely in the portal zones of connective tissue increased deposit of round cells was also seen in these zones It is uncertain however whether the inflammatory reaction is attributable to the charcoal In the kidneys a few small charcoal particles were seen in interstitial capillaries and in 2 cases in vascular loops in the glomeruli (fig 7)

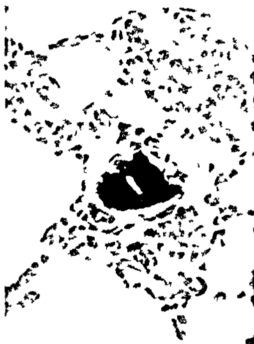


Fig 5



Fig 6

Figs 5 and 6 Massive charcoal depositions in small pulmonary arteries Haematoxylin-eosin × 120

The spleens of 10 animals showed distinct haemosiderosis Small areas of pyelonephritis were seen in 4 cases In a few lungs there were pneumonic foci or minor haemorrhages in the alveoli without any relation to the presence of charcoal One animal had parasitic granulomas in the liver, probably due to coccidiosis In 8 of the animals diffuse small inflammatory foci were seen in the cerebrum in some cases they were recent with leucocytic reaction, in others resembling epithelioid cell granulomas with a surrounding wall of round cells These lesions could hardly represent a reaction to charcoal particles but must rather have developed on an infectious basis possibly induced or activated by the treatment Electrodes for EEG had not been introduced surgically into any of these animals

Comments

Haemoperfusion through activated charcoal is a relatively new method and not much experience has so far been gained with it, either experimentally or clinically We therefore fell justified in reporting and discussing some of our observations during application of the method

Despite careful rinsing of the columns a sparse intermixture of microscopical charcoal particles was invariably noted in the blood It seems plausible that such particles should be formed continuously throughout the rinsing and perfusion procedures, as larger charcoal particles knock and rub against one another Particles of the same size as the formed elements of the blood cannot reasonably be filtered off separately One possible way of solving this problem would be to separate blood corpuscles and serum and treat the latter alone with charcoal Effective filtering might then be made and a finer charcoal powder with greater absorbing capacity could be used

Sterilization of the charcoal granulate was necessary If it was not done the animals died within about 24 hours Bacterial culture from untreated charcoal granulate yielded profuse growth of a mixed flora predominantly gram-negative rods Our method of sterilization differs from those used by Yatzidis (6) and Kolff (3) It seems unlikely that this could explain the occurrence of charcoal in the tissues of our animals Instead of sterilization by boiling we used sterilization by dry heat for 24 hours at 200 C in 2 cases and in 2 cases autoclaving for 2 hours at 120° C In none of the cases did we note any decrease in the amounts of charcoal in the rinsing fluid blood, or internal organs

The flow rate was in most of our cases 3—4 ml per minute that is 1—2 ml per kg body weight and minute In Yatzidis s perfusions in dogs and man the flow rate seems to have been at the level of 4—5 ml per kg body weight and minute



Fig 7 Charcoal deposit in a glomerular capillary loop Haematoxylin eosin × 460

The flow rate could probably have been improved in our cases if we had used a pump

The results in our phenobarbital intoxicated series can be compared with those obtained by Alwall et al (2) in 1952 They report that in a control series the animals were sitting steady 42 0 (72—24) hours after intramuscular injection of 130 mg of phenobarbital per kg body weight Rabbits treated by forced polyuria induced by administration of fluid 0 25 l per kg body weight, sat steady after 40 5 (57—24) hours and by administration of 0 50 l per kg body weight after 28 4 (36—24) hours Animals treated by exchange ultrafiltration sat steady after 12 2 (13—12) hours and by haemodialysis after 11 0 (14—9) hours With perfusion at a

good flow rate the animals in our series sat steady after 13 5 (7 8—29 8) hours Accordingly, the duration of narcosis is at about the same level as in treatment by exchange ultrafiltration or haemodialysis The deviations in the perfused group are great, which may be attributable to varying flow rates in the columns, variations in the perfusion time, the number of column changes and the particle size

The uraemic animals showed no significant clinical improvement during the perfusion The chemical changes taking place in the blood during the perfusion were in line with the observations reported earlier

The continual occurrence of charcoal in internal organs of the perfused animals demands attention It has not been reported earlier and its clinical significance cannot yet be assessed Until more is known about this phenomenon, haemoperfusion through activated charcoal should be done only in restricted cases

Summary

Haemoperfusion through activated charcoal was introduced in 1964 by Yatzidis as an alternative or a complement to dialysis treatment of endogenous and exogenous intoxications, such as uraemia and barbiturate poisoning The method has been used in experiments on animals as well as therapeutically in man and no serious complications have been noted After a short survey of the relevant literature, we present a modified perfusion technique suitable for experimental use in rabbits and describe its application in untreated in phenobarbital intoxicated, and in uraemic animals

Charcoal haemoperfusion was performed in 11 control rabbits, no complications were seen during an observation period of up to 9 months

Thirteen rabbits were given 130 mg of phenobarbital per kg body weight intramuscularly The perfusions were started after 4 to 6 hours when the animals were unconscious In 7 cases with a good flow rate the narcosis lasted for 13 5 (7 8—29 8) hours In Alwall s et al experiments in 1952 with an analogous technique in intoxicated untreated rabbits the duration of narcosis was 42 0 (72—24) hours and in rabbits treated by haemodialysis it was 11 0 (14—9) hours The treatment methods seem to be equally effective The greater range in our series could be due to varying flow rates in the columns, varying length of perfusion time, the number of column changes, and the size of charcoal particles used

In 6 rabbits the ureters on both sides were ligated 2—3 days before the charcoal haemoperfusion The animals were drowsy at the beginning of the treatment and did not improve during the course of it They died on the 4th — 5th day The results of the chemical analyses of the blood were consistent with those published earlier

The microscopical examinations revealed charcoal particles in the lungs of all the haemoperfused animals In two-thirds of the cases charcoal was also found in the spleen and in half these cases in the liver as well One fourth of the animals had charcoal particles in the kidneys In 2 cases a few particles

suspected to be charcoal were seen in the brain Only a slight tissue reaction was noted The pathogenetic significance of this charcoal deposition can not yet be evaluated

Perfusion of blood through activated charcoal leads to effective removal of certain retention products in uraemia and of phenobarbital in poisoning with this substance It seems that the method can also be successfully applied in other endogenous and exogenous intoxications However the deposit of charcoal pigments in internal organs invariably noted after the treatment calls for restriction in the use of the method in man

Acknowledgement

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From the Institute of Hygiene (Head Haakon Natvig M D) University of Oslo Oslo Norway

Studies on Hemoglobin Values in Norway

IV Hemoglobin Concentrations among School children

By

Haakon Natvig, Tor Bjerkedal and Oyvind Jonassen

Although no routine determination of the hemoglobin concentration is made in Norwegian school children a few schools medical officers have done so for short periods for particular purposes (3 4, 13 15) The results of a number of studies conducted in other Scandinavian countries (1, 8 9 11 20, 22) and many besides (2, 5 10 19) indicate that mild anemia is by no means rare among children of school age

In the present study repeated determinations of the hemoglobin concentration were performed in school children attending a number of primary schools in Oslo and Finnmark with the object of ascertaining the normal values for Norwegian school children which would provide a basis for assessing the frequency of anemia

An earlier study (16) has shown that the mean hemoglobin concentration for primary school-children at Kirkenes (70 N) was not lower than for school children in Oslo (60 N) In another study (17) it was found that the variation in the mean hemoglobin concentration over the year was from 5 to 10 per cent for school children at Kirkenes and Oslo There was however, no material seasonal variation in the levels

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Material and methods

Determinations supplementing those in the two last mentioned studies have been made at a number of other schools Thus the present material was obtained at 3 primary schools in Oslo and at 10 in Finnmark All the girls and boys enrolled in the third and fourth classes in 1960 were included in the study they ranged in age from 10 to 13 years and were thus old enough not to be apprehensive of the blood sampling and young enough for there to be no material effect of puberal changes

The data relating to the schools the children examined and the number of hemoglobin determinations performed are presented in table I

Fig 1 shows the geographical location of the various schools in Finnmark Kirkenes Vardo and Honningsvåg are located along

TABLE I Number of children examined at the various primary schools and the number of hemoglobin determinations

Primary school	Period of examination (day of month, month, year)	No of children	No of times examined	No of Hb determinations
Oslo				
Kampen	10 2 60 to 15 2 61	64– 70	13	872
Ila	5 9 to 13 12 60	73– 87	2	160
Marienlyst	11 5 to 10 12 60	47– 51	3	148
Finnmark. coast				
Kirkenes	28 1 60 to 27 1 61	144–168	11	1 628
Vardø	9 3 60 to 13 1 61	105–117	7	771
Honningsvåg	9 4 to 17 12 60	128–132	3	392
Finnmark hinterland				
Kautokeino	2 4 to 5 11 60	39– 45	2	70
Polmak	14 3 60 to 10 1 61	22– 23	4	90
Skipagurra	15 3 60 to 10 1 61	13– 15	4	56
Sirma	14 3 60 to 10 1 61	13– 18	4	62
Grensen	6 4 to 7 9 60	15– 16	2	31
Masi	5 4 to 7 11 60	18	2	36
Karasjok	7 4 to 6 9 60	10– 14	2	24
	Total	774		4 [illegible]00

the coast of Finnmark, while Kautokeino Polmak, Skipagurra, Sirma, Grensen, Masi and Karasjok, whose population is predominantly Lapp, are located in the hinterland.

The blood tests were performed at the schools. The samples were taken from the finger tip with Sera Sharp Blood Lancets

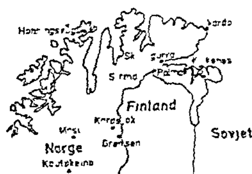


Fig 1 The geographical location of the various schools in Finnmark.

(Propper Manufacturing Co Inc N.Y.) After removal of the first few drops with cotton wool, 0.20 ml of blood was drawn into a dry adjusted pipette and immediately mixed with 3.5 ml of a 0.1 % solution of sodium carbonate.

The hemoglobin determinations were performed by means of a Linson Junior Apparatus (AB Ljungberg & Co Stockholm) 2–4 hours after the blood samples had been taken. The apparatus with its cuvettes was compared at the Central Laboratory, Ullevål Hospital, Oslo, with a Beckman DU Apparatus in which oxyhemoglobin and cyanmethemoglobin were read and the conversion factor was checked. For the conversion a millimolar extinction coefficient of 11.5 was used and the molecular weight of hemoglobin was taken as 16 520.

In Finnmark all the blood samples were taken and all the hemoglobin determinations

TABLE II Mean hemoglobin concentration and S D for boys and girls in the third and fourth classes

Classes	No of determinations	Boys mean (g/100 ml)	S D	No of determinations	Girls mean (g/100 ml)	S D	No of determinations	Both sexes mean (g/100 ml)	S D
Third	1 059	13 18	0 81	1 044	13 14	0 84	2 103	13 16	0 83
Fourth	1 141	13 24	0 88	1 156	13 15	0 77	2 297	13 19	0 83
Total	2 200	13 21	0 85	2 200	13 14	0 80	4 400	13 18	0 83

made by the same well trained health nurse In Oslo a trained school nurse took the blood samples and the hemoglobin readings were performed by one of the authors (N)

Results

Hemoglobin in relation to age and sex

The mean hemoglobin concentration for boys and girls in the third and fourth classes and the standard deviations for the respective distribution of values are given in table II

The differences in the mean hemoglobin concentrations and the standard deviations for the boys and girls of the two classes were so small that for the further analysis the groups were combined

For the whole material the mean concentration was 13 18 g/100 ml with a standard deviation of 0 83 As fig 2 shows the distribution of the hemoglobin concentration was nearly symmetrical and 95 % of all the determinations lay between 11 5 and 14 8 g %

Hemoglobin concentrations by place of residence

The mean hemoglobin concentrations for the children at the various schools in Oslo and Finnmark ranged from 13 48 to 12 56 g/100 ml the highest mean was recorded at Ila and Marienlyst schools in Oslo (table III) Values above the grand mean were obtained only at Kautokeino Skipagurra Masi Grensen and Kirkenes At Kampen school in Oslo and at Vardø Honnings våg Polmak and Karasjok the values were slightly below the mean Very low means were recorded for Sirma The variation in the means is statistically

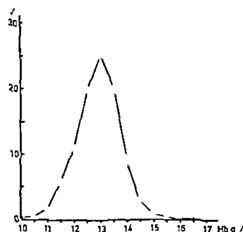


Fig 2 Frequency distribution of the hemoglobin concentrations for school children aged 10—13 years

TABLE III Mean hemoglobin concentrations and S D for school-children aged 10—13 years in Oslo and Finnmark

Primary school	No of determinations	Mean Hb (g/100 ml)	S D
Oslo			
Kampen	872	13 12	0 89
Ila	160	13 48	0 92
Marienlyst	148	13 46	0 76
Finnmark coast			
Kirkenes	1 688	13 21	0 81
Vardo	771	13 14	0 74
Honningsvåg	392	13 06	0 89
Finnmark hinterland			
Kautokeino	70	13 34	0 79
Polmak	90	13 02	0 78
Skipagurra	56	13 32	0 77
Sirma	62	12 56	0 77
Grensen	31	13 25	0 81
Masi	36	13 21	0 55
Karasjok	24	13 08	0 70
Total	4 400	13 18	0 83

TABLE IV Results of hemoglobin determinations on school children with and without supplementary iron for one year

	Supplementary iron	*No supplementary iron*
No of children	79	80
No of determinations	819	821
Mean Hb concentration (g %)	13 24	13 00
S D	0 68	0 86
Percentage with Hb < 11 5 g %	0 1	2 1
Percentage with Hb < 12 5 g %	6 4	16 9

significant both for the schools in Oslo and for those in Finnmark It would thus seem that the hemoglobin levels is dependent on the local conditions, such as social factors and diet Hence, a decision as to normal levels cannot be based on hemoglobin determinations in a group of children attending a particular school or schools

Normal hemoglobin concentrations for school children

Some of the school children aged 10—13 years who were examined had received

iron (30 mg of bivalent iron) in the form of the ferrofumarate (specially prepared for the study and supplied by Nyegaard & Co, Oslo) on each school day throughout a whole year This was given to altogether 79 children The hemoglobin values for these children were compared with those for a group of 80 children who had been given placebos over the same period Both groups were from the same school and were similar with respect to age and sex At the beginning of the experiment the two groups had the same mean hemoglobin concentration In the course of the year hemoglobin determinations were performed on each child once a month The results of this study are presented in table IV

Not only was the mean higher for the group receiving supplementary iron but the percentage of children with low hemoglobin values was much lower The effect of the iron is best illustrated by a comparison of the distributions of the hemoglobin concentrations for the two groups (fig 3) The hatched field represents the proportion of children with low hemoglobin values who got benefit from the supplementary iron intake

It would seem justified to take the findings in the group receiving adequate iron as the basis for what should be regarded a normal hemoglobin concentration for Norwegian school-children aged 10—13 years This group had a mean of 13 24 g /100 ml and the standard deviation of the distribution of values was 0 68 On the assumption that values within ± 2 5 S D from the mean are within the normal range values below 11 5 g/100

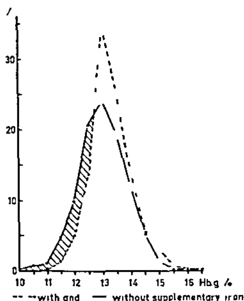


Fig 3 Frequency distribution of the hemoglobin concentrations for school children with and without supplementary iron for one year

ml would appear to indicate definite anemia In addition our findings indicate that most of the children with a value between 11 5 and 12 5 may have mild iron-deficiency anemia In these cases too supplementary iron is probably indicated

The frequency of anemia in Norwegian school-children

On the basis of the above criteria the frequency of hemoglobin concentrations below 11 5 and 12 5 g/per 100 ml was determined for the various schools The results are presented in table V

Hemoglobin values below 11 5 g/100 ml were found among school children both in Oslo and in the coastal regions and hinterland of Finnmark It was only in the group comprising the children in Kirkenes who received supplementary

TABLE \ Frequency of anem a in Norweg an school-ch ldren

Pr mary school	Percentage of Hb read ngs	
	<11 5 g %	<12 5 g %
Oslo		
Kampen	3 2	23 1
Ila	2 5	13 1
Mar enlyst	0 7	8 8
F nnmark		
Kirkenes	1 7	17 8
Vardo	1 6	17 6
Honn ngsvåg	4 3	25 0
Kautoke no Sk pagurra Masi og Grensen	1 6	14 5
Polmak S rma Karasjok	4 5	30 7

iron and the children at Marienlyst school in Oslo that there was virtually no anemia at the other schools the frequency was 1 6—4 5 % Values below 11 5 were particularly common among the children at Polmak Sirma and Honningsvåg schools in Finnmark and at Kampen school in Oslo Of the total number of hemoglobin determinations 2 3 % were below 11 5 g

The frequency of values below 12 5 g/100 ml ranged from 8 8 to 30 7 % It would thus seem that a fairly large proportion of the Norwegian school children would benefit from supple mentary iron

Discussion

The results of the present study can be compared directly only with those of studies performed with the same method and there are none such it is interesting to note however that the values obta n ed are largely in agreement with those reported earlier by Scandinavian workers (1 8 9 11 21 22) They also accord with the values for school ch ldren given in the text books (6 24) The means were higher for British (5) Canad an (2) and Australian school children (19) but whether this is due to differences in method or whether it is real is imposs ble to say

The boys of the fourth class had a slightly higher mean hemoglobin con centration than those of the third and than the girls of both classes Th s is consistent with the findings of other large studies (2 8 22 23) and is most probably due to higher values for a few boys with a particularly early physical development (2)

Relatively high hemoglobin values were found at the two west end schools in Oslo with good social conditions This may have been due to early physical development of several of these children The considerably lower means at Vardø and especially Hon ningsvag than at Kirkenes probably reflect the better social econom c and

nutritional conditions at Kirkenes The mears for the Lapp children show however, that the hemoglobin concentration need not always be influenced by economic and hygienic conditions In a few typical Lapp districts the values were higher than for Oslo and Kirkenes in spite of poorer housing and nutrition It is possible that the iron intake by these children is adequate because of the high iron content of the reindeer meat Of the particularly low values among the children at Sirma no explanation can be offered

Normal values for hemoglobin concentration are usually based on determinations for a representative sample of apparently healthy persons but even the apparently healthy can have anemia owing to inadequate supply of iron in the diet Thus it would seem that what should be considered the normal' level and what should be considered as abnormally low levels — that is, anemia — can be determined only after iron in sufficient quantity has been given over a long enough period to reliably cover the iron needs

The basis that we propose for the normal levels for Norwegian school-children would seem adequate, since the data were obtained from apparently healthy children who received a large enough dose of supplementary iron for a long enough period of time to rule out alimentary anemia The diagnosis of anemia would seem justified in school-children with a hemoglobin concentration below 11 5 g per 100 ml Values between 11 5 and 12 5 g might be normal yet frequently indicative of mild anemia Whether mild anemia exists in an individual child can in fact, be determined only by hemoglobin determinations subsequent to an adequate supply of iron Levels below 12 5 g/100 ml in school-children would therefore in general indicate the need for an iron supplement

In view of the fact that up to 3 2 % of the school children in Oslo and up to 4 5 % in Finnmark had a hemoglobin concentration below 11 5 g and up to 23 and 30 %, respectively, had a value below 12 5 g iron deficiency would seem to be a common condition among Norwegian school children Recent studies in other Scandinavian countries have also disclosed a relatively high frequency of anemia in school-children (11, 20 22) This is probably due to a low iron content of the diet (7 12 18) combined with a poorly balanced diet

The results of this study would seem to indicate the need for including routine hemoglobin determination in medical examinations of school children as was suggested by one of us as early as 1947 (14)

Summary

A total of 4400 hemoglobin determinations were performed on 774 boys and girls aged 10—13 years at 3 primary schools in Oslo and 10 in Finnmark in the course of one year

The mean hemoglobin value for all the children was 13 18 g/100 ml ± 0 83, with only an insignificant difference between the boys and girls in the third and fourth classes The mean values for hemoglobin concentration for children

from the various schools ranged from 12 56 to 13 48 g/100 ml

In 79 school-children who had received 30 mg of bivalent iron in the form of the ferrofumarate on every school day for one year, the mean hemoglobin level was higher and the proportion of low values smaller than for a control group of 80 children

On the basis of the hemoglobin concentrations in children who had received an adequate supply of iron for one year, a value of 13 2 g/100 ml ± 1 7 is to be considered normal for Norwegian school children aged 10—13 years Values below 11 5 g should be taken to indicate anemia Values between 11 5 and 12 5 g/100 ml may exceptionally be normal but ordinarily are indicative of mild anemia and of a need for iron supplements

The frequency of anemia (i e below 11 5 g/100 ml) in Norwegian school children was up to 3 2 per cent in Oslo and 4 5 per cent in Finnmark The corresponding figures for values below 12 5 g were 23 and 30 per cent

The results of the study indicate that routine hemoglobin determinations should be incorporated in the school health program in this country

Acknowledgement

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From the Institute of Hygiene (Head Haakon Natvig M D) University of Oslo Oslo Norway

Studies on Hemoglobin Values in Norway

V Hemoglobin Concentration and Hematocrit in Men Aged 15—21 Years[1]

By

Ketil Natvig

Although, according to the text books on clinical hematology, primary iron deficiency anemia in man is a rare condition (14) there is evidence from a number of studies on apparently healthy population groups that in young men the state is quite common (2 8) This has been ascribed to the particularly large iron requirements in the period of growth (2 5)

In a fairly large-scale investigation carried out in Norway it was found that among men aged 15—19 years the frequency of hemoglobin concentration below 11 8 g/100 ml was 1 2 per cent In the light of the results of diet studies showing that the iron content of the Norwegian diet fell from 17 3 mg to 12 3 mg for 3 300 calories between 1906 and 1950 (3) and that it is lower than in other countries (7), it has been considered advisable to supplement the iron intake of among others young men in the period of growth (10)

In an evaluation of the hemoglobin level account must be taken of the effect of slight hydremia and dehydration on the values In clinical work this is not a significant factor, but in studies intended to detect milder degrees of iron deficiency in the apparently healthy sector of the general population they may well prove of significance In such studies it would therefore seem necessary to consider the hemoglobin concentration in relation to the hematocrit (packed-cell volume) so that the mean corpuscular hemoglobin concentration (MCHC) can be calculated Such investigations are few and they are moreover, not very representative (6 9 13)

In the present study the hemoglobin and hematocrit levels and the MCHC were recorded for groups of apparently healthy young men aged 15—21, with the object of examining whether the levels varied with age whether there was an increase in the frequency of anemia

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The results of the study indicate that routine hemoglobin determinations should be incorporated in the school health program in this country

Acknowledgement

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TABLE II Mean hemoglobin concentrations for the various age groups

Age (yrs)	No of subjects	Mean hemoglobin conc (g/100 ml)	Standard error
15–16	36	14 64	± 0 17
17	57	14 62	± 0 13
18	55	14 61	± 0 12
19	55	14 79	± 0 14
20	51	14 70	± 0 12
21	58	14 43	± 0 12
Total	312	14 63	± 0 05

There was no significant difference between the hemoglobin values for the various age groups $t<2$

between the various participants and other factors that might affect the hemoglobin values by way of the blood volume care was taken to ensure that all the age groups were uniformly represented according to a random sequence at each sampling

Results

The age distribution of the material is shown in table II As 8 subjects in the 15 year group were to attain 16 years in a very short time they were assigned to the 16 year group

Table II also shows the mean of the *hemoglobin concentration* in the individual age groups and for all the subjects examined The means differ only slightly and there was no statistically significant difference between the highest and lowest values, t being less than 2

The standard deviation S was obtained from the expression

$$S = \sqrt{\frac{(X-M)^2}{N-1}}$$

where $(X-M)^2$ is the sum of the squares of

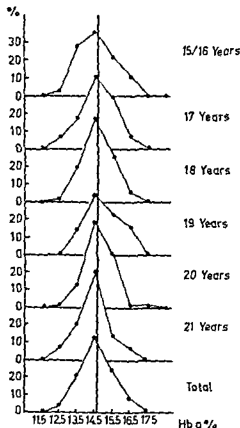


Fig 1 Frequency distribution of hemoglobin values for the different age groups The vertical line indicates the mean for the series (312 men)

the difference between the individual observation from the mean and N is the number of observations

The standard error of the mean S E is $\frac{S}{\sqrt{N}}$

$$t = \frac{M_1 - M}{\sqrt{SE_1^2 + SE_2}}$$

When $t = 2\,5$ the probability is about 99 % and when $t = 2$ about 95 % that the difference between the two groups compared is not due to chance

The shapes of the distribution curves for the hemoglobin concentration were approximately the same in the various groups (fig 1) the variations were no

TABLE III Relative frequency of hemoglobin concentrations of less than 13 0 g/100 ml and MCHC less than 30%

Age (yrs)	Percentage with Hemoglobin conc <13 0 g/100 ml	MCHC <30%
15–16	2 8	6 2
17	7 0	6 0
18	1 8	13 4
19	0	4 1
20	2 0	17 4
21	6 9	11 1
Total	3 5	9 9

TABLE IV Mean hematocrit for the various age groups

Age (yrs)	No of subjects	Mean hematocrit (%)	Standard error
15–16	32	45 25	± 0 54
17	50	45 66	± 0 41
18	52	46 65	± 0 43
19	49	46 29	± 0 39
20	46	46 63	± 0 36
21	54	45 57	± 0 41
Total	283	46 05	± 0 18

Between the 18- and 15/16 year groups t = 2 02
Between the 20 and 15/16 year groups t = 2 12
Otherwise t < 2 0

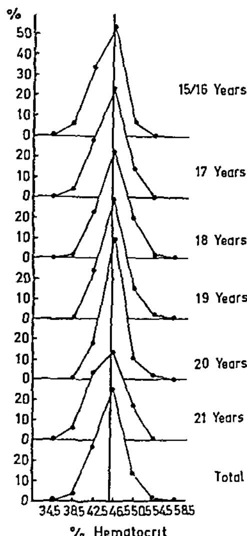


Fig 2 Frequency distribution of hematocrit values for the different age groups The vertical line indicates the mean for the series (283 men)

larger than might be expected for such a small material, and in none of the groups was there any systematic skewness towards higher or lower values

The lowest hemoglobin concentrations, namely 12 0 g/100 ml, related to men in the 17-year group The frequency of values below 13 0 g in the various age groups is given in table III

The *mean hematocrit* was relatively low in the 15/16—17— and 21-year groups and high in the 18—20 year groups (table IV) The differences were statistically significant at the 5 per cent level from the 15/16 year to the 18 and 20 year groups but not for the others Correspondingly, the distribution curves in fig 2 showed some skewness towards

TABLE V Mean MCHC for the various groups

Age (yrs)	No of subjects	Mean MCHC (%)	Standard error
15–16	32	32 41	± 0.27
17	50	32 14	± 0 24
18	52	31 38	± 0 24
19	49	31 92	± 0 21
20	46	31 64	± 0 26
21	54	31 56	± 0 21
Total	283	31 80	± 0 10

Between 15/16 and 18 year groups t=2 86
Between 15/16- and 20 year groups t=2 06
Between 15/16 and 21 year groups t=2 47
Between 17 and 18 year groups t=2 32
Between 17 and 21 year groups t=2 15
Otherwise t<2 0

lower values, especially for 15/16- and 21 year groups

The *mean MCHC* diminished from the 15/16 year to the 18 year group, the difference being significant at the 1 per cent level ($t = 2\ 86$) (table V) The other results of the t test are given in the table

The MCHC *distribution curves for* the 15/16 and 17 year groups displayed a skew towards the right — that is, towards higher values — while the 18-, and 20 and 21 year groups were distributed at a lower level than the mean (fig 3)

The relative frequency of MCHC values below 30 per cent in the various year classes is given in table III

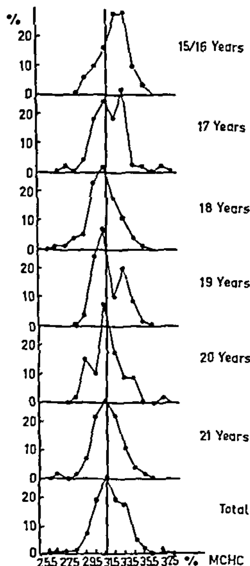


Fig 3 Frequency distribution of values of MCHC for the different age groups The vertical line indicates the mean for the series (283 men)

Discussion

The mean *hemoglobin concentration* of 14 63 g/100 ml for the material of men aged 15—21 years was higher than that found in an earlier Norwegian study on men aged 15—19 years at various industrial works throughout the country (10) However the mean level for those of the latter series who were employees in iron and other

engineering enterprises was similar to that for the present study (10, 11) The present results do not confirm the increase in the mean hemoglobin levels with age from 15 to 20 years reported by Hawkins et al (4) Nor was there any particularly low mean among the individual year classes of men aged 15—21 years There were no values below 11 8 g/100 ml, which is usually taken as the lower limit for normality in men, and has been found in 1 1 and 1 2 % of men under 21 years in earlier studies (2, 8, 10) The absence of such low values in the present study may have been due to anemia among the persons excluded from the material owing to disease or because they had received iron therapy, again many of those working in the iron and other engineering enterprises may have taken in additional iron from iron dust This is considered to account at least in some measure for the on average higher hemoglobin levels for the workers in the iron industry than for the men in other industries (10)

It is, however, open to question what hemoglobin level should be taken as the limit for anemia in men Even though values below 13 0 g/100 ml (that is, twice the standard deviation below the mean) may occur as a rare exception among normal men, they should as a rule be regarded as indicative of anemia On this basis anemia would have been present in 3 5 % of men aged 15 21 years composing the material for the present study

In 51 healthy men aged 19—30 years Linneberg and Schartum Hansen (9) found a mean *hematocrit* of 44 76 % with a range of 39—52 % Kilpatrick (6) reported 44 23 % for a group of 29 healthy men aged 15—24 and Walsh et al (13) 45 69 % for 23 men aged 16—25 years Wintrobe (14) puts the normal values at 47 ± 7 % The mean for 283 men aged 15—21 was 46 05 %, with a range that was largely the same as the recorded normal values, there being only 3 men with values below 40 and only one recording over 54 %

The mean hematocrit was slightly higher for the 18 , 19- and 20 year groups than for the others, but the differences were not statiscally significant and none of the classes showed particularly low or high values

Because the MCHC quotient is calculated from both the hemoglobin con centration and the hematocrit, the values might contain the method error for the determination of both these levels For this reason small differences in the MCHC values between the age classes should not be ascribed any significance

From the tables published by Linneberg and Schartum Hansen (9) for 51 healthy men aged 19—30 years the mean MCHC was found to be 34 85 %, with a range of 31 3—39 1 (calculated by the present author)

Wintrobe (13) gives 34 ± 2 % as the normal MCHC, and states that values below 30 % are indicative of hypochromia For 29 men aged 15—24 years whom Kilpatrick (6) examined the mean MCHC was 31 76 ± 0 79 % This is in close agreement with the mean found for the present series of 283 men aged 15—21 years, namely 31 80 ± 0 10 % In view of the fact that 9 9 % of the subjects had MCHC

values of less than 30 % while the lowest value for the earlier Norwegian material (9) of men aged 19—30 years was 31 3, the present mean of 31 80 suggests the presence of hypochromia, particularly among the 18-year olds, for whom the MCHC was significantly lower than for the 15/16 year group It is seen from fig 4 that the low MCHC value for the 18-year-olds was due to the relatively high hematocrit

Whether the MCHC is a more sensitive indicator of mild iron deficiency anemia than the hemoglobin concentration alone is difficult to decide on the basis of the present findings It is true that values of MCHC below 30 %, which may, of course be considered as indicative of hypochromia were considerably more common in the present series of 15—21 year subjects than were hemoglobin values below 13 0 g/100 ml, it is, however, difficult to say whether the low MCHC signifies that there was mild iron deficiency anemia

A reliable criterion of iron deficiency anemia in a group of healthy subjects is that the blood level responds to the supply of iron By recording the hemoglobin concentration and the hematocrit and calculating the MCHC after giving supplementary iron, it can be ascertained whether there was iron deficiency This has been the object of a new study performed at this Institute

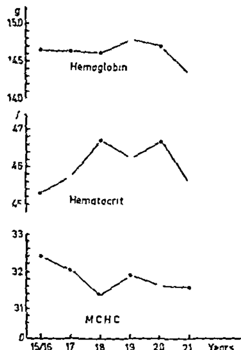


Fig 4 The means for the hemoglobin hematocrit and MCHC in the various age groups

Summary

In 312 healthy men aged 15 21 years employed in the iron and other engineering enterprises in Oslo a mean hemoglobin level of 14 63 g 100 ml was found there was no evident difference between the individual year classes as regards the means or distribution None of the subjects had values below 12 0 g but 3 5 per cent had less than 13 0, which may be considered to be a more realistic limit for what shall be regarded as anemia in young men

The mean hematocrit was 46 per cent with slightly lower values in the 15/16, 17 and 21 year than in the 18—20-year groups

The mean MCHC for all the men aged 15—21 was relatively low, at 31 8 per cent and about 10 per cent of the subjects had values below 30 Whether this is indicative of hypochromia and whether the MCHC is a more sensitive indicator of mild iron

deficiency anemia than the hemoglobin concentration alone might be ascertained by recording the MCHC for a group given supplementary iron

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Acta Medica Scandinavica Vol 180, fasc 5, 1966

From the Institute of Hygiene (Head Haakon Natvig M D) University of Oslo Oslo Norway

Studies on Hemoglobin Values in Norway

VI Hemoglobin Concentration, Hematocrit and MCHC in 19 year old Men

By

Oivind Larsen

The major Norwegian investigations on normal values for hemoglobin and hematocrit have been performed on office and industrial employees (11 13 15), students (6 10) and school children (12—14) The object of the present study was to record the hemoglobin concentrations and hematocrit and thus the MCHC, for a single age group of men in a particular geographical region in Norway so as to derive the normal levels and hence a measure of the incidence of anemia for this group

Material

The material consisted of apparently healthy men born in 1946 in 1965 they were residing in the county of Ostfold and were summoned for a military medical examination at the 1965 session

Of those attending the session 72 men were examined by other medical officers and were therefore not included in the study Owing to technical mishaps in the blood sampling 9 were discarded, the final series thus consisted of 1 624 men or 81 1 % of the total age group of Ostfold males (18)

Submitted for publication May 23 1966

Those not attending the session fall into the following groups

1 Men suffering from such diseases that their medical classification could be recorded without their attending the regular session Hence no men with serious illnesses were included in the material

2 Men residing temporarily outside the county or who owing to temporary absence were prevented from attending the session in their own district at the correct time This group contained relatively more seamen than the age class as a whole

3 Men not attending the session in their own district or not attending in the year they were called up owing to temporary illness or accidents

As a social description of the material it can be said that about 91 % of the men examined were living at home with their parents as members of the household about 44 % were engaged in industry craft work or related occupations and about 35 % were still at school

Methods

All the men composing the series underwent the ordinary clinical medical examination which was performed on 95 5 % of the subjects by the author For each subject a

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TABLE I Hemoglobin concentration in 1 624 19 year old men

Hemoglob n (g/100 ml)	No	%
< 10 9	2	0 12
11 0–11 4	2	0 12
11 5–11 9	0	0 00
12 0–12 4	2	0 12
12 5–12 9	4	0 25
13 0–13 4	7	0 43
13 5–13 9	19	1 17
14 0–14 4	84	5 17
14 5–14 9	157	9 67
15 0–15 4	309	19 03
15 5–15 9	373	22 97
16 0–16 4	289	17 80
16 5–16 9	211	12 99
17 0–17 4	102	6 28
17 5–17 9	24	1 48
18 0–18 4	27	1 66
18 5–18 9	8	0 49
≥ 19 0	4	0 25
Total	1 624	100 00

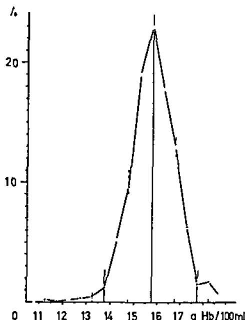


Fig 1 Percentage distribution of hemoglobin concentration for 1 624 men aged 19 years

special form was completed with name military number occupation home environ ment and relevant case history

From each man blood samples were taken by a specially trained member of the medical corps who determined the hemoglobin level hematocrit and MCHC

Capillary blood was taken from the tip of the fifth finger after cleaning with disin fectant After removal of the first drop 0 20 ml of blood was taken for the hemoglobin determination and added to 3 5 ml of Drab kin s liquid consisting of 1 000 g of sodium bicarbonate 0 200 g of potassium ferro cyanide ($K_3Fe(CN)_6$) 0 050 g of potassium cyanide and water to 1 000 ml the pipette was rinsed with the reagent After at least 30 min the color of the solution was read in a Linson Junior photoelectric colorimeter (AB Lars Ljungberg & Co Stockholm) with use of a green filter and square cuvettes The apparatus was standardized against a cyanmethemoglobin solution (British Drug Houses Ltd) and the extinction was converted to grammes of hemoglobin per 100 millilitres correct to the nearest tenth of a gram For technical and practical reasons it was impossible to include duplicate determinations in the procedure and as a check of the method independent blood samples were taken from both fifth fingers of 81 randomly chosen men (5 0 % of the series) With the specified level of accuracy there was no difference in the two measure ments for 73 of the subjects (90 1 %) in 5 persons the difference was 0 1 g/100 ml while 3 persons showed differences of 0 5 1 0 and 1 4 g/100 ml

For determination of the hematocrit a drop of blood was drawn into a heparinized capillary tube which was sealed at one end and centrifuged in a hematocrit centrifuge (AB Lars Ljungberg & Co Stockholm) at 8000 rev /min for 10 min The percentage of red corpuscles was read directly from a nomogram to the nearest one per cent In none of 81 duplicate determinations was there any difference in the two values

TABLE II Hematocrit in 1 624 19 year-old men

Hematocrit (%)	No	%
<34	1	0 06
35	2	0 12
36	3	0 18
37	10	0 61
33	10	0 61
39	11	0 68
40	33	2 03
41	35	2 16
42	71	4 37
43	112	6 90
44	187	11 52
45	253	15 58
46	247	15 21
47	217	13 36
48	154	9 48
49	111	6 84
50	92	5 67
51	45	2 77
52	20	1 23
53	5	0 31
54	1	0 06
55	4	0 25
>56	0	0 00
Total	1 624	100 00

From the values recorded, the MCHC (the number of gram of hemoglobin per 100 ml of packed red blood corpuscles) was determined from the formula

$$MCHC = \frac{\text{hemoglobin (g/100 ml)}}{\text{hematocrit (\%)}} \times 100$$

The examinations were performed in the period 21st April to 20th August 1965 but no account was taken of the time when during that period the specimen was taken

At the outset the lower limit for normal hemoglobin value was arbitrarily chosen at 12 8 g Hb/100 ml and all the men with this or lower values were when possible subjected to further examination at the Department of Internal Medicine Central Hospital for Ostfold Fredrikstad (Head Ole K Evensen

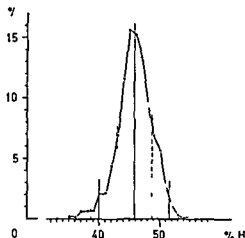


Fig 2 Percentage distribution of hematocrit for 1 624 men aged 19 years

M D) A few subjects were referred to the nearest laboratory for an out patient examination of the blood Because the men attending the session are usually not called up for military service for 1 or 2 years it was not possible to arrange a follow up by the military medical service, and it had therefore to be arranged on a voluntary basis for this reason it was not so complete as desired

Results

The mean hemoglobin concentration for the 1 624 subjects was 15 75 g/100 ml (rounded up to 15 8 g/100 ml), S D 1 00 g/100 ml (table I, fig 1)

The mean hematocrit was 45 8 %, S D 2 9 % (table II, fig 2)

The mean MCHC was 34 42 % S D 1 62 % (table III fig 3)

Previous illness

Seventy nine of the subjects (4 9 %) reported earlier illnesses that might have affected the blood values Of these 29 complained of dyspepsia of varying degree but the hemoglobin hematocrit

TABLE III MCHC in 1 624 19 year-old men

MCHC (%) (g Hb/100 ml of erythrocytes)	No	%
< 27 9	3	0 18
28 0—28 9	4	0 25
29 0—29 9	12	0 74
30 0—30 9	33	2 03
31 0—31 9	67	4 13
32 0 32 9	193	11 89
33 0 33 9	369	22 72
34 0—34 9	349	21 49
35 0—35 9	305	18 78
36 0—36 9	168	10 35
37 0—37 9	55	3 39
38 0—38 9	28	1 72
39 0 39 9	19	1 17
40 0—40 9	10	0 61
41 0—41 9	5	0 31
42 0 42 9	1	0 06
> 43 0	3	0 18
Total	1 624	100 00

and MCHC values for these subjects did not differ significantly from the means for the series

The other 50 reported various other illnesses, e g infections and renal disease, but since all were regarded as clinically healthy at the examination they were included in the series

Previous iron therapy

Fifty (3 1 %) had taken iron tablets in the last 12 months but usually only for quite short periods because they felt abnormally tired or were pale

Confirmed cases of anemia in the series

Nine men (0 6 %) were found to have hemoglobin values not exceeding 12 8 g/100 ml, the usual limit for anemia Of these, however, 7 had MCHC values higher than 30 0 %, the usual lower limit for normochromia

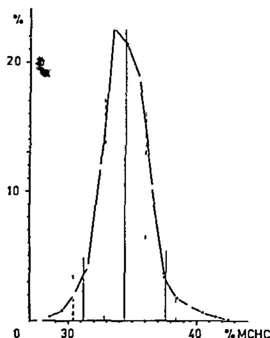


Fig 3 Percentage distribution of MCHC for 1 624 men aged 19 years

Case 1

At a secondary school living at home At 12—14 years he had constant sore throat and occasional spring lethargy ' At examination hemoglobin 12 8 g/100 ml hematocrit 37 %, MCHC 34 6 % Iron tablets were prescribed At an outpatient blood examination 8 weeks later hemoglobin 85 % (12 6 g/100 ml) hematocrit 43 % and blood smears showed a large variation in the size and shape of the erythrocytes and a tendency to hypochromia The regularity of the iron medication could not be checked and the origin of the demonstrated anemia was not obvious MCHC fell to 29 3 which in any case was indicative of a pathologic condition

Case 2

Office worker, living at home Polyarthritis since 1954 for which steroid treatment had been given The health records showed hemoglobin 90 % for the 3 last years When in autumn 1964 he was admitted to the local hospital for duodenal ulcer and anemia the level was 45 % Since leaving hospital he had taken about 200 iron tablets According to the health book the hemoglobin level in June 1965 was 85 % (12 6 g/100 ml) at the session examination in Aug 1965 it was 12 8 g/100 ml with hematocrit 38 % and MCHC 33 7 % He was extremely lean and of slender build and had diastolic murmurs over the heart No further examinations were carried out The anemia was probably due mainly to the ulcer disease

Case 3

Secondary school pupil living at home Hemoglobin 12 8 g/100 ml hematocrit 39 % MCHC 32 7 % No history or signs of anemia No further examination was made

Case 4

Pupil at a trade school living at home Hemoglobin 12 0 g/100 ml hematocrit 39 % MCHC 30 8 % No further symptoms or signs of anemia At an outpatients examination a week later hemoglobin 12 1 g/100 ml red corpuscles 5 03 mill /mm³ white cells 5 600 mm³ ESR 9 mm/1h Blood smears showed mild anisomicrocytosis with a varying saturation of erythrocytes as in mild hypochromic microcytemia No further examination was made There was probably iron deficiency anemia most likely alimentary in type

Case 5

Farm labourer living at home He was tall and lean 194 cm, 66 kg Hemoglobin 11 4 g/100 ml hematocrit 37 % MCHC 30 8 % Admitted to Department of Internal Medicine At the examination he reported some apathy and orthostatic dizziness Blood smears showed a few schizocytes and slight hypochromia There were no signs of malabsorption Iron therapy resulted in a satisfactory increase in the hemoglobin concentration The iron deficiency anemia was probably alimentary

Case 6

Lived at home Six weeks before the examination he stopped working at a nickel workshop owing to a rash There were numerous pea sized scars and scratched bleeding lesions on the trunk and limbs He was mentally peculiar and seemed to be retarded Hemoglobin 11 2 g/100 ml hematocrit 37 % MCHC 30 3 % On admission he stated that during the last few weeks he had been having mild dyspepsia had felt listless and suffered from breathlessness on exertion and anorexia The hemoglobin was then 10 7 g/100 ml blood smears showed slight anisocytosis and predominant orthochromia Low serum iron (23 γ/100 ml) and transferrin high were suggestive of iron-deficiency anemia The 24-hour excretion of fat in the feces was on the high side (9 5 g) the glucose loading curve was flat Schilling test normal There was hypersensitivity for nickel and formalin Iron therapy had a favorable effect on the anemia which was apparently due to constant small losses of blood and possible malabsorption

Case 7

Pupil at a trades school living at home He had no symptoms and was healthy in appearance Hemoglobin 12 4 g/100 ml

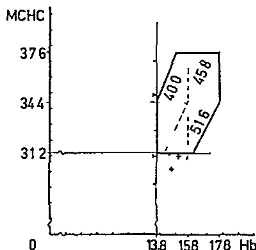


Fig 4 Correlation graph for the hemoglobin hematocrit and MCHC The MCHC is presented as a function of the hemoglobin concentration and hematocrit and the hexagon is bounded by the lines for 2 S D difference from the means (broken lines) such that about 95 % of a normally distributed material will fall within the limits The subjects with hemoglobin and/or MCHC values below the mean less 2 S D are plotted in the graph to illustrate the incidence of anemia and/or hypochromia in the examined material

hematocrit 41 % MCHC 30 2 % On admission he stated he was drinking as much as 3 litres of milk daily Iron therapy before admission apparently accounted for a serum iron level of 53 γ/100 ml and transferrin 294 γ/100 ml Blood smears showed slight anisocytosis with a few schizocytes and orthochromia with a slight hypochromia There was no evidence of malabsorption and the anemia was possibly due to a deficiency of iron in the diet for instance *the milk may have replaced* part of the food having a higher iron content

Seventeen of the men had MCHC values less than 30 0 % but normal' hemoglobin (above 12 8 g/100 ml) and of these 14 had levels greater than the mean minus twice the standard deviation, that is, 13 8 g/100 ml All 17 were healthy and only 3 of them had a history of mild anemia

Two men (0 1 %) had hemoglobin levels below 12 8 g/100 ml and also an MCHC below 30 0 %

Case 8

A secondary school pupil living at home Hemoglobin 10 6 g/100 ml hematocrit 38 % MCHC 27 9 % Because of school examinations he could not be admitted to the Department of Internal Medicine until one month later He stated he had had periodic dyspepsia for 2 years probably melena 2 years before admission and melena just before admission On admission radiography showed duodenal ulcer and the hemoglobin level was 8 0 g/100 ml This increased satisfactorily on iron therapy

Case 9

Agricultural student who was working for a time at home on the farm He periodically felt somewhat tired and listless but in the case history there were no other symptoms of anemia Hemoglobin 8 5 g/100 ml hematocrit 30 % MCHC 28 3 % On admission hemoglobin 8 4 g/100 ml later 9 5 g/100 ml ESR 16 and 12 mm/1h Blood smears showed moderate anisocytosis with schizocytes and marked hypochromia The serum iron was extremely low — 19 γ/100 ml — and the transferrin elevated at 486 γ The fecal fat excretion was elevated while the xylose vitamin A and the Schilling tests showed values on the low side The blood sugar curve after peroral administration of glucose was low He received iron parenterally because it seemed to have little *effect when given by the oral route* and he also received vitamin B_{12} A practically symptom free though significant anemia — discovered by chance — was the only sign of the probable presence of sprue

Normal values

On the basis of the present findings the normal values for 19 year old men would appear to be the following

Hemoglobin 15 8 g/100 ml ± 2 S D or 13 8 –17 8 g/100 ml
Hematocrit 45 8 % ± 2 S D or 40 0— 51 6 %
MCHC 34 4 % ± 2 S D or 31 2— 37 6 %

Fig 4 shows the range and distribution of hemoglobin, MCHC and hematocrit values and the correlation between them On either side of the lines for the means the lines for the 2 S D from the means have been drawn A material satisfying the requirements of a normal distribution with respect to hemoglobin, MCHC and hematocrit will lie with about 95 % of the subjects within the limits of the 2 S D hexagon, if the observed values are plotted in the correlation graph

Presence of anemia

Thirty two (2 %) of the 19 year old men examined had hemoglobin values below 13 8 g/100 ml If 31 2 % is taken as the limiting MCHC for hypochromic anemia 60 (3 7 %) of the group had this condition Forty eight (four fifths) of these, however had normal hemoglobin levels

Discussion

Material

For the interpretation of the results, it must be remembered that the material included a selected group of apparently healthy men from an age group where good health would usually be expected Therefore the present values more likely approximate to the normal values for healthy subjects than to the mean values which could be expected for other population groups

Accuracy of methods

The duplicate determinations disclosed a high degree of accuracy in the hematocrit determination In the hemoglobin determination, if duplicate measurements had been generally performed differences greater than 0 1 g/100 ml could be expected in approximately 60 subjects or only 3 7 per cent of the series

The revealed distribution of hemoglobin and hematocrit values thus include not only abnormal findings due to pathological conditions, but also incorrect results caused by errors of the method, the duplicate samples, however, show the unimportance of these errors

The MCHC values may — owing to their indirect determination in this study — include methodological errors from both hematocrit and hemoglobin measurements, and this must especially be considered when evaluating the clinical importance of abnormally high or abnormally low MCHC values in subjects where the findings have not been confirmed by additional examinations

Hemoglobin concentrations

Because of difference in method and the composition and size of the materials it is difficult to compare the present results with those of the earlier studies on hemoglobin levels Moreover it is difficult to establish criteria for a normal material (15) and to decide what should be taken as a normal range

As 19 year olds usually compose part of a larger material the number of such men in the investigations most suitable for comparisons is

much smaller than was examined here With few exceptions (2, 6) the mean hemoglobin values for such series were lower than for the present one (1, 3, 5, 7, 10, 11, 15, 17, 19)

Hematocrit

Wintrobe (20) gave 47 ± 7 per cent as normal for the hematocrit The value found for the present study was 46 ± 6 per cent Where the number of subjects has been mentioned in previous studies, it has always been less than in the present study (16)

MCHC

Because it is only in recent years that the MCHC has come to be used in clinical work, there are only few studies dealing primarily with this parameter The MCHC tends to fall with age (—0 031 % per annum) (8) Wintrobe (20) gives 34 ± 2 per cent as the normal level, for the 19 year old men of the present series the value was 34 4 ± 3 2 per cent

Compared with the few studies with which a direct comparison can be made (4, 10, 15, 16) the present values are slightly higher, and considerably higher than for the 15 21-year male group from an industrial workshop in Oslo (15)

Incidence of anemia

For calculating the incidence of anemia among the 19 year old men 13 8 g/100 ml was taken as the lower limit for the normal hemoglobin concentration A WHO study group (21) gives 14 g/100 ml as the lower limit for men, 36 of the present series (2 2 %) had levels below 14 g/100 ml

Sixty (3 7 %) of the men had MCHC below the lower limit, but four fifths of these had a normal hemoglobin level Whether a low MCHC is an indication of iron deficiency anemia, possibly latent, cannot be decided from the present results, but could be decided from studies of the hemoglobin level and MCHC in subjects receiving controlled adequate iron over an extended period

In the 32 men with a hemoglobin level below 13 8 g/100 ml the mean MCHC was 32 2 per cent, that is, in the lower part of the normal range

In the present context Leonard's study on 4,221 Royal Air Force recruits is of special interest (9) In 50 men who had passed grade 1 of the military medical examination, routine photo metric hemoglobin determinations dis closed hypochromic anemia which in 47 was regarded as idiopathic", and they were admitted to hospital for further examination Clinical signs were rare even in the most severe grades of anemia, but almost all the recruits felt better after iron therapy

The hypochromic anemia in the 17 subjects of the present series with an MCHC below 30 0 per cent would hardly have prompted them to consult a doctor, and in any case it is unlikely that the anemia would have been recognized so long as the hemoglobin values remained within the normal range The anemia was probably for the most part alimentary in origin

In the case of the 7 subjects with normochromic anemia it is doubtful whether the condition would have become clinically manifest at the level obtaining

The 2 reported subjects with low hemoglobin and low MCHC show how mild the symptoms of anemia can be Here, the routine examination disclosed 2 serious cases where treatment was urgent, and illustrates the value of a blood examination in, for instance a medical examination of military personnel, office and industrial workers and the like

A blood examination as performed in the present study is so simple that there should be no difficulty in fitting it into most routine examination programmes

Summary and conclusions

In an examination of 1,624 apparently healthy men of 19 years, constituting 81 1 per cent of the men of this age class in the county of Ostfold in Norway, the mean hemoglobin concentration, hematocrit and MCHC were 15 75 g/100 ml, 45 8 and 34 42 per cent respectively

Among the followed up subjects with too low hemoglobin or MCHC there was usually a pathologic condition with few symptoms Hemoglobin and MCHC levels lower than the mean less twice the standard deviation will usually indicate a pathologic condition calling for closer examination

Hemoglobin and MCHC levels below these limits were found in 2 0 and 3 7 per cent of the subjects

It is possible that the MCHC provides a means of detecting iron-deficiency anemia where the hemoglobin levels are normal and this might be confirmed in a study in which subjects with too low MCHC are given controlled iron therapy

A careful determination of the hemoglobin and hematocrit is so rapid and easy to perform that there should be no difficulty in fitting it into routine examinations of large groups for example military recruits and company personnel where it would provide an extremely valuable adjunct to the clinical evaluation

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From the Department B of Internal Medicine (Head Johs Clausen M D) The County and City Hospital of Odense, Odense Denmark

Propranolol (Inderal) in Cardiac Arrhythmias

By

B Lyager Nielsen and F Schonau Jorgensen

Since 1962 reports concerning the treatment of cardiac arrhythmias by means of beta receptorantagonists have been published with increasing frequency Generally nafsylyt (pronethanol = alderlin) has been used but this drug seems to have been relinquished in favour of propranolol (Inderal) which is also produced in the I C I laboratories

In our department propranolol has been administered to a number of patients with arrhythmias of different aetiologies The present paper is introduced by a review of the action of beta receptorantagonists but since little clinical experience has hitherto been gained, we feel it might be of interest to give an account of our therapeutic results

Chemistry and resorption

Propranolol (1 isopropylamino-3- 1 naphthyloxy) propan 2-ol hydrochloride) is chemically related to isoprenaline Upon oral application it is rapidly resorbed from the intestine 90 per cent being resorbed within 30 min Being decomposed in the liver it is rapidly excreted and therapeutic doses of the drug have been found to be eliminated in the course of about six hours (6 7)

Propranolol is available as tablets of 10 and 40 mg) and in ampoules of 5 ml 1 mg/ml) for intravenous injection

Action

Studies throughout the last 50 years on the action of secale preparations and catechol amines have suggested that in the adrenergic nervous system there are two types of receptors which selectively respond to inhibition as well as stimulation by medicaments 1

Alpha receptors have been demonstrated in the muscles of blood vessels specifically in skin and kidneys but they are present also in uterine and intestinal muscles In the dilator muscle of the pupil this type of receptor is present exclusively

Beta-receptors are present in the smooth muscles of the blood vessels specifically in striated muscles in the bronchial uterine and intestinal muscles and in the myocardium Stimulation of the myocardial beta receptors induces an increased tonus and accelerated heart rate

The effects obtained by stimulation of the

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TABLE I Effects obtained by stimulation of receptors in the adrenergic system, together with examples of known stimulants and receptor antagonists

	Stimulation of alpha receptors	beta receptors
Effect	Vasoconstriction especially in skin and kidneys Mydriasis Contraction of uterine muscles	Vasodilation especially in striated muscles Relaxation of bronchial muscles Relaxation of uterine muscles Increased myocardial tonus accelerated heart rate
Stimulants	Nor adrenaline adrenaline	Isoprenaline adrenaline
Antagonists	Ergotoxin bensylyt	Nafsylyt propranolol (Inderal)

two receptor systems are tabulated in table I together with a review of a series of known stimulants and antagonists

Propranolol should be considered a specific beta receptorantagonist which by a blocking of the beta receptors will antagonize the myocardial respon e to catecholamines and decrease the myocardial irritability

Sympathetic nerve stimulation or administration of catecholamines is known to increase the oxygen consumption by the heart to such an extent that myocardial ischaemia may ensue Since some patients have been found to exhibit an increased catecholamine concentration in blood during episodes of angina pectoris (21) there may be theoretical grounds for seeking a favourable effect through a drug induced beta receptorblockade in these cases Quite naturally the most copious reviews of the clinical effects obtained by beta receptorantagonists include accounts of its value also in cases of angina pectoris (3 10 16)

Cardiac arrythmias induced by digitalis represent another main field of therapy The arrythmia caused by digitalis preparations may entail liberation of catecholamines (22) and the explanation of the favourable effect obtained in these cases by reserpin amongst other agents may be that reserpin reduces the catecholamine concentration in the myocardium (28) This accords with the fact that tolerance to digitalis increases after sympatectomy (9) and administration of beta receptorantagonists (33)

Examination of anaesthetized patients seems to suggest that the catecholamine concentration in blood is increased during anaesthesia (20), and that certain gas anaesthetics especially halothane (WHO), intensify the myocardial sensitivity to catecholamines (4) It has been observed in several studies that favourable results may be obtainable through use of beta adrenergic blocking agents in cases of complicating extrasystoles, ventricular tachycardia and ventricular fibrillation in anaesthetized patients (5, 15, 19 24)

Finally, propranolol may also possess an anti arrhythmic action not associated with its beta receptorblocking properties (14, 26) The drug has been used successfully in the treatment of tachycardias and arrhythmias not induced by digitalis (5 12, 24 25 27) of hypertrophic subaortic stenosis (13) of thyrotoxicosis (34), and of Adam Stoke s syndrome in children due to tachyarrhythmia (31 32) In patients with phaeochromocytomata the drug has been used both as a diagnostic means (18) and combined with bensylyt (phenoxybenzamine) which is known to block alpha receptors, in the preoperative therapy Finally Snow (26) used propranolol in the prophylaxis of arrythmias in patients with acute coronary occlusion

TABLE II Therapeutic results

No	Sex	Age	Indication for propranolol	Dose	Effect
1	♂	54	AHD Series of ventricular extra systoles No response to digitalis, chinidine and procainamide	10 mg × 3 daily	Sinus rhythm restored 86/min within 24 hrs
2	♂	83	AHD Previous myocardial in farction Aortic stenosis Con gestive heart failure R BBB (Wilson type) 85/min with ven tricular extrasystoles No response to digitalis	10 mg × 4 daily	Sinus rhythm restored 56/min within 24 hrs R BBB (Wilson type) Recurrence of extra systoles after withdrawal of propranolol
3	♀	72	AHD Congestive heart failure Marked ventricular extrasysto lism about 48 extrasystoles/min	10 mg × 4 15 mg × 4 daily	Ventricular bigemina within 24 hrs No response to digitalis + propranolol
4	♂	74	AHD Marked ventricular extra systolism about 32 extrasystoles per min No response to digitalis and procainamide	10 mg × 4 daily	Rate of extrasystoles reduced within 24 hrs (20/min) Very few extrasystoles during digitalis + propranolol therapy
5	♂	61	AHD Marked ventricular extra systolism about 25 extrasystoles per min	10 mg × 4 daily	Rate of extrasystoles reduced within 24 hrs (12—20/min) No further reduction after digitalis + propranolol
6	♀	82	AHD Digitalis intoxication. Hy perthyroidism Sinus tachycardia 120/min supraventricular extra systoles No response to thiama zol (thycapzol) and digitalis involving intoxication	10 mg × 3 daily	Sinus rhythm restored 85/min within 24 hrs Several recurren ces after withdrawal of propran olol
7	♀	74	AHD Digitalis intoxication Bronchogenic carcinoma L BBB atrial fibrillation 120—200/min ventricular extrasystoles No response to digitalis involving intoxication	14 mg intravenously	Sinus rhythm 80/min L-BBB Effect during injection
8	♀	71	AHD Paroxysmal supraventric ular tachycardia 170/min in spite of digitalis and chinidine therapies	4 mg intravenously	Sinus rhythm restored 66/min Effect during injection
9	♂	65	AHD Paroxysmal supraven tricular tachycardia 225/min in spite of digitalis and chinidine therapies Daily episodes	8 mg intravenously 10 mg × 4 daily	Sinus rhythm restored 96/min Effect 8 min after injection Following a 13-day therapy with daily doses of 40 mg of pro pranolol an episode of ventric ular tachycardia occurred
10	♂	55	Bronchogenic carcinoma with ex tension to pericardium Atrial fibrillation about 200/min No response to digitalis	10 mg × 3 20 mg × 3 daily	Atrial fibrillation about 120/min Effect within 24 hrs No further reduction in ven tricular rate after increased doses

Table II Cont

No	Sex	Age	Indication for propranolol	Dose	Effect
11	♂	56	AHD Auricular flutter 170/min No response to digitalis chinidine procainamide and gilurytmal (ajmalin)	15 mg × 4 daily	Atrial fibrillation 60—100/min Effect within 24 hrs Following 1 week treatment with digitalis + propranolol sinus rhythm 80/min was restored
12	♂	70	AHD Congestive heart failure Atrial fibrillation 100—150/min with supraventricular extra systoles No response to digitalis	10 mg × 4 15 mg × 4 daily	No response to a 1 week treatment with a daily 40 mg of propranolol During combined digitalis + propranolol therapy (60 mg) atrial fibrillation 75—100/min
13	♂	61	AHD Auricular flutter 72/min during digitalis therapy	10 mg × 4 15 mg × 4 daily	Auricular flutter 64/min Effect within 24 hrs
14	♀	64	RHD Atrial fibrillation 80—100/min	10 mg × 4 daily	No response
15	♀	61	AHD Paraxysmal atrial fibrillation about 150/min Episodes occurred during lanatosid and chinidine therapies No tolerance to procainamide Prior to propranolol therapy 11 episodes within 21 days Normal sinus rhythm 72—82/min in intervals between episodes	10 mg × 4 10 mg × 5 15 mg × 4 daily	Initially during treatment sinus rhythm Daily episodes of atrial fibrillation during treatment with 40 and 50 mg of propranolol per day After doses of a daily 60 mg of propranolol 1 episode within 10 days In intervals between episodes sinus rhythm about 50/min
16	♂	64	AHD Recent myocardial infarction Ventricular tachycardia 175/min Daily doses of digitalis	10 mg × 4 daily	Sinus rhythm 75/min Effect within 24 hrs
17	♂	65	AHD Episodes of paroxysmal ventricular tachycardia 170/min	8 mg intravenously 10 mg × 4 daily	No response The attack subsided after procainamide
18	♂	56	AHD Ventricular fibrillation	5 mg i v	No response Death
19	♂	57	AHD Acute myocardial infarction Ventricular fibrillation	10 mg i v	No response Successful DC defibrillation after antazoline Later death occurred
20	♂	71	AHD Acute myocardial infarction For 3 months prior to the coronary occlusion the patient had received daily doses of digitalis and 60 mg propranolol because of atrial fibrillation and rapid ventricular rate Following occlusion ventricular fibrillation	5 mg i v	Asystolism immediately upon injection Following cardiac massage irregular ventricular rhythm about 120/min Death ensued

AHD = arteriosclerotic heart disease RHD = rheumatic heart disease R BBB = right bundle branch block L BBB = left bundle branch block

Therapeutic results

Diagnoses indications for propranolol therapy, dosages, and the results obtained are tabulated in table II It should be noted that most patients were suffering from arrhythmias which had failed to respond to the conventional treatments, inter alia, digitalis, chinidine and pro cainamide

Side effects

Following a two month treatment with propranolol mental depression developed in patient no 1, but the symptom sub sided after withdrawal of therapy Extrasystoles recurred

In patient no 2 the blood pressure became reduced to 115/60 mm Hg within the initial 24 hours, followed by a normalization of values to about 140/80 mm Hg In two patients (nos 8 and 9) the systolic blood pressure was seen to fall by 30 and 50 mm Hg, respectively immediately upon intravenous injection of propranolol

In five patients GOT and LH en zymatic values showed a moderate (dubious) rise, but became normal in all cases during continued treatment

Discussion

Four patients (nos 14 17 18, 19) failed to respond to treatment with propra nolol the therapeutic result obtained in patient no 12 must be considered rather dubious Otherwise it can hardly be doubted that the results were attribut able to propranolol By oral application the effect would occur within 24 hours after intravenous application the effect would set in during or immediately after injection In several cases the arrythmias would recur after withdrawal of therapy and subside again as soon as the treatment was resumed

Among patients with ventricular extrasystoles the effect of propranolol would be most clear cut in cases in which the arrhythmia was secondary to digitalis in toxication, although improvement was seen also in four out of five patients who did not present symptoms of intoxication Thus the results obtained in the present investigation seem to be superior to those reported by other authors (5 11, 23)

Six patients (no 7 and nos 10—14) were suffering from chronic atrial fibril lation Sinus rhythm was restored in two of these and, in addition the ven tricular rate became satisfactorily re duced in three others In conformity with reports in the literature, reversion to sinus rhythm was rare, while adequate ventricular rates generally were achieved (5, 11, 23)

In one patient with frequent episodes of paroxysmal atrial flutter the drug seemed to be of prophylactic value

In two patients exhibiting paroxysmal supraventricular tachycardia attacks were cut by injections of propranolol Ginn et al (11) treated seven patients presenting paroxysmal atrial tachycardia secondary to digitalis in five all of whom reverted to normal sinus rhythm In spite of prophylactic propra nolol therapy however an attack of ven tricular tachycardia developed in pa tient no 9 In five patients with paroxysmal supraventricular tachycardia, Bes terman and Friedlander (5) did not find the drug an efficient prophylactic agent

The therapeutic value of propranolol in cases of ventricular tachycardia remains to be defined Besterman and Friedlander (5) have obtained favourable results from the treatment of five attacks, in three patients, of this type, which is in contrast to findings obtained by Rowlands et al (23) who noted merely an abbreviation of the episodes In the two patients discussed in the present paper, sinus rhythm was restored in one while the other failed to respond to the drug

Three patients suffering from ventricular fibrillation received intravenous propranolol Two of these patients did not respond, and in the third asystolism ensued after injection These results are inferior to those obtained by others Sloman et al (25), for instance, achieved sinus rhythm in two out of three patients, and despite the failure in preliminary experiments, DC defibrillation proved successful in the third Similarly, Besterman and Friedlander (5) achieved sinus rhythm in one patient exhibiting ventricular fibrillation induced by overdoses of isoprenaline

In contradistinction to findings when nafsylyt had been used (2, 17) it has not been possible in animal experiments to demonstrate any carcinogenic action of propranolol (30) Side effects of propranolol have been found to include insomnia, erythema, nausea vomiting, diarrhoea, dizziness, paraesthesia, mental depression, irritability, raised serum urea levels, raised GOT- and LH enzymatic values falls in blood pressure following intravenous application, aggravation of existing bronchial asthma and heart failure

In the present series, treatment had to be discontinued in one patient because of side-effects (mental depression) Falls in blood pressure were noted in three patients, and in five the GOT- and LH enzymatic values were found to be elevated Thus, in general the treatment has been well tolerated and the majority of patients had no inconveniences from the therapy, this accords with previous experience (10, 16, 29)

Manifestations of bronchial asthma were not seen in any of the patients In four patients exhibiting congestive heart failure and treated with diuretics, the incompensation did not become worse during the propranolol treatment

Conclusion

As in previous reviews (5, 11, 12, 23 24, 27) the present material is of a limited nature, and final conclusions can hardly be drawn as regards the therapeutic applicability of beta receptorantagonists in cases of arrhythmias

Propranolol was found to be effective in patients with ventricular extrasystoles, particularly if induced by digitalis, and in cases of paroxysmal supraventricular tachycardia In most patients with atrial fibrillation/flutter, either sinus rhythm was restored, or the ventricular rate was satisfactorily reduced

Two patients out of three with ventricular fibrillation failed to respond to the propranolol therapy, immediately upon injection of the drug asystolism occurred in the third

In one case the therapy had to be discontinued because of the development of mental depression No side

effects were observed except in three patients who experienced falls in blood pressure, and in five in whom the GOT and LH enzymatic values were found to be temporarily elevated

Summary

An account is given of the action of beta receptorblocking drugs and of the results obtained in 20 patients with different types of arrhythmias treated with propranolol

Regular sinus rhythm was restored in four patients out of seven presenting ventricular extrasystoles in two cases the frequency of the extrasystoles was reduced

Six patients were suffering from chronic atrial fibrillation/flutter In two of these sinus rhythm was restored in three the ventricular rate became satisfactorily reduced In one patient with paroxysmal atrial flutter propranolol proved to have a prophylactic effect

In each of two patients with paroxysmal supraventricular tachycardia the propranolol therapy was effective

In one out of two patients exhibiting ventricular tachycardia normal sinus rhythm was restored after administration of propranolol whereas in three patients with ventricular fibrillation the therapy was unsuccessful

Certain hazards may be involved in a propranolol treatment of ventricular fibrillation since asystolism occurred in one patient immediately upon injection

Among the 20 patients subjected to the treatment, mental depression was seen to develop in one three patients experienced falls in blood pressure and in five the GOT- and LH enzymatic values were found to be temporarily elevated

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From the Department of Internal Medicine II (Head E Wassén M D) and the Department of Obstetrics and Gynecology (Head S Brody M D) University of Goteborg Goteborg Sweden

Menstrual Blood Loss and Iron Deficiency

By

LEIF HALLBERG, ANN MARIE HOGDAHL, LENNART NILSSON and GORAN RYBO

The iron balance in the body is normally maintained by increasing the absorption of iron from the diet to compensate for increased iron losses The absorption is limited by the amount of dietary iron and its absorbability It is not definitely known how much iron can be absorbed from an ordinary diet by normal subjects but the amount probably varies between 0 5 and 2 0 mg daily (18)

The variation in the iron losses from the body is even more difficult to ascertain, especially in women due to the menstrual iron losses In an earlier study of the menstrual blood loss in 12 young, healthy women it was found that the menstrual blood loss was very constant from one period to another, but *that there were marked differences between* subjects (13) The individual constancy of the menstrual blood loss observed in these young subjects suggested that a confirmation of this observation in a larger series of women with a wider age range would lead to a more complete understanding of the critical iron balance situation in women The iron losses in women are comprised mainly of menstrual blood losses and losses due to desquamation of epithelial and endothelial cells The amount of iron lost by desquamation is probably fairly constant, i e about 0 6 mg daily in adult females (6) The individual variation in the total iron losses is thus probably mainly due to the variation in the menstrual iron losses between different women

The purpose of the present study was thus 1 to extend the previous studies on the constancy of the menstrual blood loss to a larger series comprising women of different ages and 2 to study the relationship between the menstrual *blood loss and parameters reflecting the* iron state of the body

Material and methods

The study comprises 137 women working in a factory and was performed during the autumn of 1962 in connection with a voluntary health screening The women were be

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TABLE I Parity in different age groups The first figures indicate the number of subjects, and the figures within brackets indicate the number of subjects in per cent of the total number in each age group

No of children	Age group				
	$<$25 years	26—35 years	36—45 years	$>$46 years	Total
0	38 (79)	12 (28)	8 (20)	0 (0)	58 (42)
1	7 (15)	10 (23)	13 (32)	1 (20)	31 (23)
2	3 (6)	13 (30)	9 (22)	0 (0)	25 (18)
$>$2	0 (0)	8 (19)	11 (27)	4 (80)	23 (17)

tween 16 and 52 years old (mean age 30 2 years) In 117 women two consecutive menstrual periods were studied in 20 only one period The mean duration of menstruation in the series was 5 3 ± 0 14 days while the mean interval between menstruations was 28 8 ± 0 35 days based on the subjects own statements To examine the influence of age the following division of the series into age groups was made

Group 1 48 women up to 25 years mean age 20 3

Group 2 43 women 26—35 years mean age 30 3

Group 3 41 women 36—45 years mean age 39 4

Group 4 5 women more than 45 years mean age 48 6

The last group was formed to exclude a possible influence of the climacterium on the menstrual blood loss The small number of subjects in this group makes it impossible to come to valid conclusions as to the magnitude of blood losses in women above 45 years Therefore, statistical comparisons comprised only groups 1 2 and 3

The parity of the women in the different age groups is given in table I Of the women up to 25 years 79 per cent were nulliparous in the other age groups 78 per cent had born children

The examination comprised a general medical past history with detailed recording of the menstrual pattern a brief social and dietary history and measurements of height and weight A venous blood sample was drawn for hematological analyses

Every woman was examined gynecologically Uterine fibroids were found in 4 and ovarian cysts in 2 subjects In the other 131 women the gynecological examination did not reveal any pathological findings

The menstrual blood loss was determined according to Hallberg and Nilsson (12) i e alkaline hematin was determined in a sodium hydroxide extract from simultaneously used towels and tampons Hemoglobin was determined using the cyanomethemoglobin method Plasma iron was determined according to Bothwell and Mallet (4) and total iron binding capacity (TIBC) according to Bothwell et al (3)

Results

The mean menstrual blood loss of the first period studied in all 137 subjects was 34 0 ± 2 4 ml The mean value of the first period in the 117 subjects studied during 2 periods was 33 9 ± 2 5 ml The mean menstrual blood loss of the second period in these 117 subjects was 34 5 ± 2 6 ml The difference between the mean values of the first and second period in the 117 subjects was not statistically significant An analysis of variance also showed that the dif

TABLE II Analysis of variance of two consecutive measurements of the menstrual blood loss in 117 women

Variance due to	Degrees of freedom	Variance	F	P
Individuals	116	1 409 4	11 7	P <0 001
Periods	1	19 61	0 2	P >0 2
Rest variance	116	120 4		

TABLE III Menstrual blood loss and hematological values at different ages Mean values and standard error of means in different age groups The figures within brackets indicate the number of subjects

Age group	Menstrual blood loss (ml)	Hemaglobin conc (g/100 ml)	MCHC (per cent)	Plasma iron (μg/100 ml)	Total iron binding capacity (μg/100 ml)
<25 years	28 9±1 94 (48)	13 5±0 15 (48)	33 0±0 33 (48)	106±5 1 (46)	323±5 2 (44)
26—35 years	35 4±3 83 (43)	13 8±0 13 (43)	33 8±0 27 (43)	88±4 4 (39)	318±6 6 (36)
36—45 years	34 1±5 46 (41)	13 6±0 19 (41)	33 3±0 30 (41)	103±7 8 (40)	328±7 8 (38)
>46 years	75 0±25 00 (5)	12 2±0 94 (5)	32 8±1 39 (5)	72±10 6 (5)	336±15 1 (5)
Total	34 2±2 35 (137)	13 5±0 10 (137)	33 4±0 18 (137)	98±3 4 (130)	324±3 6 (123)

ference between the periods was not significant, but that the difference between women was statistically significant (see table II) The standard deviation between the periods was 4 4 ml The mean menstrual blood loss in the entire series was 34 2 ± 2 4 ml This value is based on the mean values in the 117 women measured twice and the single values in the other 20 women

The distribution of the menstrual blood loss in the first period (137 subjects) is shown in fig 1 A comparison of the distributions in the first and second periods in the 117 subjects measured twice is shown in fig 2 The median value was 26 2 ml The blood loss was less than 86 8 ml in 95 per cent of the women The mean menstrual blood loss in groups 1—4 was 28 9 35 4, 34 1 and 75 0 ml respectively (table III) In group 4 comprising 5 women more than 45 years old, 2 women had very heavy losses (114 ml and 153 ml) No statistically significant differences exist between age groups 1—3

The distribution curves shown in fig 3, however indicate a shift towards

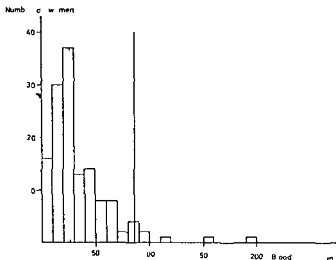


Fig 1 Distribution of menstrual blood loss in 137 subjects (first period) The median value is graphed as a dotted line and the 95th percentile value as an unbroken line

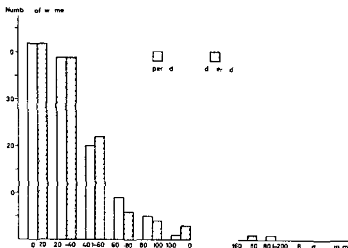


Fig 2 Comparison of distribution of menstrual blood loss in 117 subjects measured twice

higher values in groups 2 and 3 compared with group 1 The skewed distribution is also illustrated by the lower 95th percentile value in group 1 (58.3 ml compared with 93.4 ml in each of the other two groups)

As shown in table I there was a great difference in parity between group 1 and the other groups The low mean value and low 95th percentile value observed in group 1 were thus not necessarily due to an effect of age as such but may as well have been an effect of other factors related to age for instance parity

The mean menstrual blood loss for the 58 nulliparous women was 29.7 ± 2.3 ml for the 31 women having one child 28.1 ± 3.2 ml for the 25 having two children 35.6 ± 5.2 and for the 23 women having more than two children 58.7 ± 9.5 ml

However as parity is related to age it is necessary to study the effect of parity in the separate age groups No

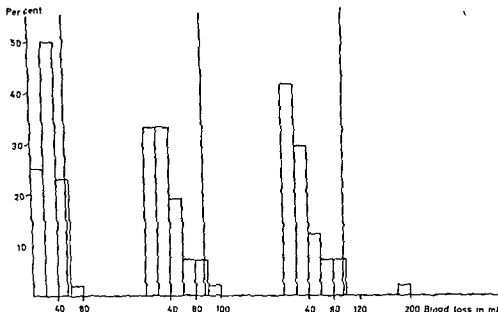


Fig 3 Distribution of menstrual blood loss in age groups 1 2 and 3 (from left to right) The vertical lines indicate the 90th percentile values in the groups

systematic influence of parity on the magnitude of the menstrual blood loss was then found except for women 25 years or younger in whom the 10 parous women had a menstrual blood loss of 42 0 ±5 3 ml compared with 27 4 ± 1 9 ml for the 38 nulliparous women This difference was statistically significant ($p < 0\ 01$) This finding may indicate that a more recent delivery may increase the menstrual blood loss

Hematological data

Mean values (with their standard errors) for the hemoglobin concentration the mean corpuscular hemoglobin concentration (MCHC) the plasma iron concentration and the total iron binding capacity (TIBC) are given in table III for the total material and the different age groups It is evident that the mean values were almost the same in all age groups except in the oldest, in which lower values of hemoglobin, MCHC and plasma iron were observed However

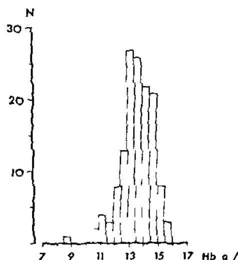


Fig 4 Distribution of hemoglobin values (g/100 ml)

TABLE IV Hematological data in relation to menstrual blood loss Mean values and standard errors of mean The figures within brackets indicate the number of subjects

Menstrual blood loss (ml)	Hemoglobin conc (g/100 ml)	MCHC (per cent)	Plasma iron (μg/100 ml)	Total iron binding capacity (μg/100 ml)
0–20	13 7±0 14	34 1±0 33	101±1 3	319±5 7
	(47)	(42)	(46)	(45)
20 1–40	13 6±0 15	34 0±0 27	103±4 4	318±5 8
	(50)	(50)	(46)	(40)
40 1–60	13 1±0 24	33 0±1 77	90±6 4	320±8 7
	(21)	(21)	(20)	(20)
60 1–80	13 6±0 29	33 7±0 81	83±9 8	342±13 9
	(10)	(10)	(9)	(9)
80 1–100	13 9±0 24	35 0±0 80	78±12 0	359±19 3
	(6)	(6)	(5)	(6)
>100	10 8	31 8	43	383
	(3)	(3)	(3)	(3)

ever, this group comprised only 5 subjects The distribution of the hemoglobin values for the whole material is shown in fig 4 Only ten subjects (7 3 per cent) had a hemoglobin concentration below 12 g/100 ml

The relationship between menstrual blood loss and hematological findings

Subjects with heavy menstrual blood loss may sooner or later develop an iron deficiency Pregnancy has also a marked effect on the iron balance and may lead to an iron deficiency anemia if adequate iron prophylaxis is not given The probability of such an anemia will be higher with increasing number of pregnancies When studying the relationship between menstrual blood loss and hematological data reflecting the iron state of the body, it is necessary to consider the effect of parity on the relationship As mentioned before, there was no systematic influence of parity on the magnitude of the menstrual blood loss A further analysis has also shown that there was no influence of parity on the hematological data studied, except for women 25 years or younger in whom the hemoglobin concentration was lower in the parous women (12 8 g/100 ml) than in the 38 nulliparous women (13 6 g/100 ml) ($p < 0\ 01$) The lack of systematic influence of pregnancies on the hematological data may be explained by the general prophylactic use of iron during previous pregnancies in this series as reported by the subjects

The results of this analysis reduce the probability of a systematic error in the study on the relationship between menstrual blood loss and hematological values

The mean values (with their standard errors) for hemoglobin and plasma iron concentration, MCHC and TIBC

for different ranges of menstrual blood loss are given in table IV The MCHC and the hemoglobin concentration were lower in the three women having a menstrual blood loss exceeding 100 ml In women with smaller losses these values were unrelated to the magnitude of the loss The plasma iron concentration decreased with increasing menstrual blood loss starting in the range 40—60 ml The TIBC showed an increase with increasing loss starting in the range 60—80 ml (fig 5)

As pointed out previously only 10 out of 137 subjects had a hemoglobin concentration below 12 g/100 ml blood In these 10 women the mean menstrual blood loss was 58 0 ml compared with 32 5 ml for the others The difference was statistically significant ($p < 0.02$) In the 42 women having a plasma iron concentration below 80 μg/100 ml plasma the mean menstrual blood loss was 42 4 ml compared with 30 5 ml for those having a plasma iron concentration ⩾ 80 μg/100 ml plasma The difference in menstrual blood loss between the two groups was statistically significant ($p < 0.05$) The mean menstrual blood loss for the 5 per cent of the series having the highest TIBC values was 50 9 ml compared with 30 4 ml for the other 95 per cent The difference in menstrual blood loss between the two groups was statistically significant ($p < 0.005$)

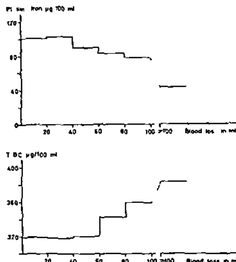


Fig 5 Total iron binding capacity in plasma (TIBC) and plasma iron concentration in relation to menstrual blood loss

Discussion

The mean menstrual blood loss in the present series was 34 ml This loss is of the same magnitude as the mean menstrual blood loss in most previously reported series (cf 11) In the earlier series, the mean value varied between 25 and 37 ml In four series (1, 2 7 17), however, considerably higher mean values were observed (45—70 ml) Two of these series comprised only a few subjects (7 17) In the other two series, subjects were accepted as normals when the hemoglobin concentration exceeded 10 2 and 10 4 g/100 ml respectively (1, 2) Thus subjects with anemia were included in these series which may explain why there was a higher mean menstrual blood loss than in the present study

In one of these series which was reported in detail (2) the median value (35 9) was also higher than the median value observed in the present series (26 2 ml) The blood loss range was 6 6—178 7 ml compared with 1 6—199 7 ml in the present series These figures

show that there was a smaller proportion of subjects with heavy menstrual blood loss in the present series than in the other ones

The frequency of anemia in the present series was small — only 7 3 per cent had a hemoglobin value below 12 g/100 ml This frequency is considerably lower than that observed in the general female population from which the present series originated (9) It was also lower than in population studies in England (15 16) Also this fact implies that the menstrual blood loss in the present series cannot be considered representative for the blood loss in the general population However, the purpose of the present study was not primarily to study the variation in the menstrual blood loss between different individuals, but to study the individual constancy of the menstrual blood loss and the relationship between menstrual blood loss and iron deficiency The fact that the present series was not representative for the general population will thus not interfere with these studies

Individual constancy of menstrual blood loss

In the present series, the menstrual blood loss was measured during two consecutive periods in 117 subjects The finding by means of an analysis of variance that the difference between periods was small and not statistically significant is in agreement with the results of a previous investigation of 12 women studied for 12 consecutive periods (13) In earlier investigations in which repeated periods were studied, no statistical analysis was made of the individual variation in the menstrual blood loss The main reason was probably that the series were considered too small to allow such an analysis The opinion of earlier investigators of the menstrual blood loss on the individual variation has differed greatly (cf Hallberg et al (10)) The individual constancy of the menstrual blood loss has not previously been established

The present confirmation of the individual constancy of the menstrual blood loss in a large series has certain important practical implications Thus, a single determination of the menstrual blood loss may be a fairly good expression for the average blood loss in a woman Moreover, the immediate effect of various treatments such as medication surgery etc on the menstrual blood loss may easily be evaluated quantitatively by measurements in consecutive periods The fact that there was no systematic difference between the first and the second period further indicates that the women found the present sampling technique easy to use

Menstrual blood loss and iron deficiency

Another implication of the individual constancy of the menstrual blood loss is related to the iron balance in women The menstrual loss of iron is a main factor affecting the iron balance in women The smallness of the individual variation in the menstrual blood loss from one period to another observed in this study, as also found for 12 consecutive periods in a previous study (13) together with the present observation that there was no systematic change in the magnitude of the menstrual blood loss with age except for women 46 years

or older indicate that the individual menstrual blood loss is probably fairly constant from the menarche almost up to the menopause The variation in the menstrual blood loss between different individuals may thus be a measure of the variation of the iron need between individuals, since other losses of iron, for instance through desquamation of epithelial and endothelial cells may be considered fairly constant in healthy women

The results indicate that the marked effect of pregnancy on the iron balance in women may have been counteracted by the general use of adequate iron prophylaxis during pregnancy in the present series This fact makes the variation in the menstrual blood loss between women very important for the iron balance The other main variable determining the iron balance in women i e the dietary intake of iron, was not studied in the present series A short dietary history was included in the study mainly to verify that there were no serious divergences in dietary habits In the present series, 40 subjects had two main meals and 97 one main meal a day The mean menstrual blood loss and the mean values of the hematological data were the same in subjects having one and two meals a day The importance for the iron balance of variations in dietary intake of iron in women deserves more detailed studies than those made in the present series

The present interpretation of the effect of the individual constancy of the menstrual blood loss on the iron balance indicates that the probability that a subject will develop an iron deficiency is a function of the size of the menstrual blood loss and of time (number of menstruations), no account being taken of variations in intake of iron with the food or through medication The fact that there was an increased TIBC in plasma and a decreased plasma iron concentration with increasing menstrual blood loss thus supports this conclusion The study also shows that during the development of an iron deficiency, changes of plasma iron and TIBC precede the decrease in the hemoglobin concentration, that was seen only in the small group in the present series having losses exceeding 100 ml per period These observations are thus quite consistent with the results of Conrad and Crosby (5) in their studies of the hematological effects induced by repeated phlebotomies in healthy male volunteers In their studies, the earliest sign of iron depletion was a decreased plasma iron and an increased iron binding capacity of plasma Similar findings were reported by Hagberg (8) in studies on blood donors

The amount of menstrual blood which could be lost in the present series without inducing sign of iron depletion was about 40—60 ml If the average hemoglobin value in women having menstrual blood losses below 40 ml is reckoned to be 13 6 g/100 ml and the menstrual interval to be 29 days a menstrual blood loss of 40 ml corresponds to a daily loss of 0 6 mg iron while a blood loss of 60 ml corresponds to a daily loss of 1 0 mg iron With the addition of the basic losses of iron by desquamated cells — about 0 6 mg/day (6) — the total critical daily loss

TABLE V Hemoglobin concentration and menstrual blood loss before and during treatment in 10 patients

Subject	Effect of iron therapy: Hemoglobin concentration (g/100 ml) Before	During	Menstrual blood loss (ml) Before	During
1	9.7	12.7	360	382
2	11.1	12.9	88	98
3	11.0	13.7	202	192
4	8.0	11.6	69	190
5	10.4	12.0	125	171
6	9.7	13.0	97	89
7	10.9	12.0	130	154
8	10.8	13.9	129	152
9	10.1	12.2	131	109
10	9.9	12.3	240	269

of iron will be 1.2—1.6 mg The average dietary daily intake of iron in women at the corresponding ages in the same population was found to be about 10 mg (10) This means that to maintain iron balance at this range of menstrual blood loss an average of 12—16 per cent of the dietary iron must be absorbed The calculations show that the critical range of the menstrual blood loss is in very good agreement with other more or less well established data concerning the iron balance

In a study on the dietary intake of iron in women at different ages in Goteborg it was found that the average daily intake decreased successively from 11.5 mg in the 15 year old to on an average 9.3 mg in the 45 and 50 year old women (10) If this fairly constant iron intake is put together with the individual constancy of the menstrual blood loss, it can be expected that women with a blood loss exceeding 40—60 ml per period as a rule cannot build up a body store of iron Further implications are that the larger the menstrual blood losses, the sooner an iron deficiency anemia will develop and the more marked will the anemia be before the decreasing menstrual iron losses due to the anemia, will balance the absorption of iron In this general discussion, the individual variations in dietary intake of iron were disregarded

An important point in the analysis of the relationship between iron deficiency and menstrual blood loss is the problem of cause and effect The menstrual blood loss was studied in 10 patients with iron deficiency anemia and heavy menstrual blood loss before and during treatment of the iron deficiency

The mean blood loss after treatment was slightly higher than before treatment (table V) This finding is thus

consistent with the report of Jacobs and Butler (14) It may thus be concluded that menorrhagia is not caused by iron deficiency anemia

The low frequency of anemia in the present study indicated that the menstrual blood loss might be larger in the general population This assumption was confirmed in 476 women from the same population selected at random in whom the mean blood loss was 43 4 $\pm$ 2 3 ml (10) compared with 34 2 $\pm$ 2 4 ml in the present series However the mean blood loss in 183 of the women of the general population sample who considered themselves healthy and having normal menstruations was 33 2 $\pm$ 1 6 ml This value was thus the same as that observed in the present series of working women This may indicate that the magnitude of the menstrual blood loss is of importance for the fitness for work as the frequency of non healthy subjects in the population sample was statistically higher in the group having abnormal than in the group having normal menstrual blood loss (11)

As stated in the introduction, there is incomplete information on the size of the main factors determining the iron balance in the body The evident effect on the iron balance of the observed variations in the menstrual blood loss between individuals due to the individual constancy of the blood loss indicates that information on the variation in the iron need of the general population can be obtained by studying the variation in the menstrual blood loss in a random sample of the population Such data may make possible a calculation of the expected frequency of iron deficiency in women of the population Moreover, a firmer basis to calculate a suitable dietary intake of iron will be obtained

Summary

Menstrual blood loss was determined in 137 women, in 117 of these for two consecutive periods An analysis of variance showed that the individual variation in menstrual blood loss from one period to another was small, but that the variation was great between the subjects

The mean menstrual blood loss was the same in age groups up to the age of 45 With increasing menstrual blood loss, there was an increase in the total iron binding capacity of plasma and a decrease in plasma iron These changes occurred in women with a blood loss exceeding about 60 ml per period The hemoglobin concentration and the mean corpuscular hemoglobin concentration (MCHC) were reduced only in a few subjects with a menstrual blood loss exceeding 100 ml per period

Because of the individual constancy of the menstrual blood loss observed, knowledge of the variation in the menstrual loss of iron in different women makes it possible to calculate the variation in the average iron need in women

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From the Isotope Division, Department of Alcohol Research, Karolinska Institutet Stockholm Sweden

Iron Absorption and Serum Erythropoietin Concentration in Patients with Cancer of the Uterine Cervix

By

D Lockner

Iron absorption is influenced by the rate of erythropoiesis (2 4 10 16, 22, 24), the amount of iron contained in the organism (3, 5, 8 18—20) and the degree of anaemia (17) Studying the mechanisms causing anaemia in patients with cancer of the uterine cervix, the author found moderate anaemia and an increase in the effective and possibly also the ineffective erythropoiesis (11, 14 15) He therefore investigated whether such changes influence erythropoietin concentration in serum and iron absorption in such patients

Methods

The control cases consisted of patients suspected to have cancer of the uterine cervix but showing only benign erosions The cancer patients suffered from cancer of the uterine cervix classified according to Kottmeier (9) By careful case history patients with abnormally high sanguinous discharges exceeding normal menstruation were excluded

Iron absorption was determined according to Saylor and Finch (23) Known amounts of two different iron radioisotopes were given one per os and the other intravenously From the relation of the isotopes contained in the erythrocytes one to several weeks after administration compared with the relationship of the amounts of radioiron given the percentage of iron absorbed from the oral dose can be calculated Such studies were performed about 6 p m immediately after the patients dinner In a syringe containing 80 μc Fe^{55} citrate (spec act 15—30 mc/mg Fe) 20 ml of venous blood was aspirated and the contents slowly reinjected 65 μc Fe^{59} chloride (spec act 15—30 mc/mg Fe) 1 mg Fe as $FeCl_3$ and 20 mg ascorbic acid were diluted with 70 ml tap water in a plastic covered paper container The mixture was drunk by the patient immediately after the injection of Fe^5 The container was rinsed with about 30 ml water which was also given to the patient.

Approximately three weeks after the application of the radioiron venous blood was obtained from the patient and the content of both Fe^{59} and Fe^{55} determined As radioiron three weeks after application is contained practically exclusively in the erythrocytes measurements were performed on whole blood haemolyzed by freezing and thawing The Fe^{59} content was determined by means of a well type NaI (Tl) scintillation counter The Fe^{55} content was measured and discrimi

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TABLE I Blood values from control and cancer patients and percentage Fe^{59} incorporation (including standard error of the mean) in groups of 5—6 polycythaemic mice treated with the patients plasma

No	Stage of cancer	Hct (%)	Hb (g%)	Serum iron (μg%)	Reticulocytes (%)	Fe^{59} incorporation (%)
1	Control	35	11 1	130	2 0	2 2±0 6
2	Control	40	13 3	121	1 1	4 1±0 7
3	Control	41	13 2	225	1 6	1 2±0 3
4	Control	35	11 3	124	0 9	4 6±1 3
	Mean	38	12 2	150	1 4	3 0±0 7
1	II B	37	11 9	47	0 3	10 9±2 6
2	II B	43	13 3	63	1 2	3 1±0 5
3	II A	36	12 6	186	1 5	0 5±0 1
4	II B	38	13 1	202	1 1	0,8±0 2
5	II B	42	13 6	128	1 4	4 4±0 7
6	III IV	33	10 8	87	1 2	13 9±3 1
	Mean	38	12 6	119	1 1	5 6±1 2

nation of the radiations from Fe^{55} and Fe^{59} was performed by means of liquid scintillation counting in an EKCO counter as recently described by the author (13) The percentage of iron absorbed from the oral dose of Fe^{59} was calculated as described by Saylor and Finch (23)

Erythropoietin concentration in unprocessed serum was determined by measuring the stimulation of Fe^{59} incorporation into the erythrocytes when given to polycythaemic mice The animals used were NMRI mice having a mean weight of about 25 g and fed with mouse pellets (Anticimex No 210) and water ad libitum These were made polycythaemic by injecting suspensions of mice erythrocytes in saline containing about 80 % erythrocytes at a dose of 0 5 ml Patient serum was won after spontaneous coagulation of the blood sample obtained The administration to the mice was started the same day During the test the rest of the serum was kept refrigerated Each serum was tested in 6 animals All injections were given intraperitoneally The following scheme was applied Erythrocyte suspension was given on days one to four seven and nine Test plasma was administered on days seven to ten radioiron on the tenth day 0 5 to 1 0 μc Fe^{59} was applied as citrate diluted with saline in a volume of 0 1 ml and of a specific activity as described above The animals were killed on the 13th day and venous blood was obtained The blood volume of the mice was determined simultaneously using Cr^{51} labelled mouse erythrocytes as described by the author (12) From the radioiron content of the whole blood and the blood volume determined the total amount of radioiron contained in the circulating erythrocytes was calculated Three days after radioiron injection the radioactivity is practically completely contained in the erythrocytes The total amount of radioiron contained in the circulating erythrocytes was expressed as the percentage of the amount of radioiron injected

Haemoglobin was measured as cyanmethaemoglobin in a Zeiss PMQ II spectrophotometer using Acuglobin (Ortho Pharmaceutical Corp USA) as a reference stand

TABLE II Blood values and percentage of iron absorbed (including standard error of the mean) when giving Fe^{59} orally in patients with cancer of the uterine cervix

No	Stage of cancer	Hct (%)	Hb (g%)	Iron absorption (%)
1	II A	38	12 1	1 7
2	II A	47	14 9	32 5
3	I A	39	12 2	14 0
4	II B	41	12 9	0 4
5	II A	42	13 7	1 6
6	III IV	32	9.2	0 6
7	I B	42	12 7	21 7
8	II B	37	11 4	9 6
9	I B	41	13 1	5 4
10	II B	35	10 9	15 7
	Mean	39	12 3	10 3±3 38

ard Haematocrit was determined as microhaematocrit in capillaries Serum iron was measured according to Ramsay (21) using Labtrol (Dade Reagents USA) as a reference standard The percentage of reticulocytes among 1 000 red cell was determined on slides after staining one part of blood with one part of a one per cent brillant-cresyl blue solution in saline

Results and discussion

The results obtained when determining the concentration of erythropoietin in plasma of control and cancer patients are shown in table I The blood values show only minor differences between the groups studied Radioiron incorporation into erythrocytes of mice treated with cancer plasma was irregular and was not statistically significant from that of controls (0 2>p>0 05) Two cancer patients (Nos 1 and 6) showed high radioiron incorporation These patients had the lowest haemoglobin and plasma iron values of the cancer group Two control cases had similarly low haemoglobin concentrations, but normal serum iron and normal iron incorporation The findings obtained in the two cancer patients could be due to a relative iron deficiency It could, however, be shown that cancer per se reduces serum iron concentration (15) We therefore have to conclude that no influence of cervical cancer on erythropoietin concentration could be demonstrated in this study

This result is in agreement with the findings of Goltner and Friederici (7) and Biezunski et al (1) who could not observe changes in erythropoietin concentration in patients with cancer Goltner and Friederici (7) who studied patients with the same type of cancer as presented here, showed that, when bleeding was sparse and the haemoglobin concentration above 9 0 g% no clear increase in erythropoietin concentration could be demonstrated When bleeding was the main cause of the

haemoglobin reduction, erythropoietin concentration increased These results agree with the conclusion drawn by the author (15) that bleeding is not the cause of the changes in erythropoiesis observed in these patients and that the dilution anaemia often observed in them (15) does not stimulate erythropoiesis by way of an increase in erythropoietin concentration (cf 6)

The correlation between the patients haemoglobin concentration and percent age iron incorporated into mice in the present material was not significant ($0.2 > p > 0.05$ $r = -0.628$) This means that either the test used is not sensitive enough to detect smaller changes of erythropoietin concentration or that factors other than erythropoietin regulate erythropoiesis in this range of haemoglobin concentration (cf 25)

Table II shows the results obtained when measuring iron absorption in another group of patients with cancer of the uterine cervix The values show great variation Haemoglobin concen tration and iron absorption show no significant correlation with each other ($p > 0.2$ $r = -0.427$)

When a meal containing 4 6 mg Fe was labelled with $Fe^{59}Cl_3$ and given to healthy individuals, an iron absorption of 1—16 % with a mean of 5 3 % was found by Pirzio Biroli et al (19) using the same test method The iron con tent of the meal eaten by our patients is taken to be of similar size as in the study of the authors cited (19)

The difference between the mean values found by Pirzio Biroli et al (19) and that obtained in the cancer cases studied here is not significant ($p > 0.2$) Hence the present result does not in dicate increased iron absorption in pa tients with cancer The finding excludes also bleeding as the cause of anaemia in these cancer cases, as iron absorption is known to be increased in such cases (5) The results obtained are in agreement also with those obtained by Mendel (16), who found that the stimulation of solely extramedullary erythropoiesis in mice does not influence iron absorption The increase in erythropoiesis observed in patients with cancer of the uterine cervix is partly due to ineffective erythropoiesis and the production of short living erythrocytes (14), changes similar to those observed when extramedullary erythropoiesis is stimulated in organisms usually producing erythrocytes in the bone marrow The increase in effective erythropoiesis which is observed as well seems not to be sufficient to cause a significant increase of iron absorption Mice with much larger tumours and more pronounced anaemia were found to increase iron absorption (12)

Summary

Serum erythropoietin concentration was determined in gynaecological controls and in patients with cancer of the uterine cervix Radioiron incorporation into the erythrocytes of polycythemic mice was used as test procedure Erythropoietin concentration in serum from cancer pa tients did not differ significantly from that of controls

Radioiron absorption was measured in patients with cancer of the uterine cervix by means of the double isotope

method Iron absorption did not differ from healthy individuals studied by others be means of the same method

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From the Departments of Medicine (Head P Bechgaard M D) Surgery (Head P Holm Nielsen M D) and Diagnostic Radiology (Head Aa Wagner M D) Aarhus Amtssygehus Aarhus Denmark

Long-term Prognosis in Medically Treated Peptic Ulcer

A Clinical, Radiographical and Statistical Follow up Study

By

EINAR KRAG[1]

Owing to its frequent occurrence, chronic course and life threatening complications ulcer disease has been a subject of intensive research for more than 100 years

Among the newer analyses of the frequency of the disease may be mentioned that reported by Alsted (1), who obtained his data from questionnaires sent to all general practitioners in Denmark in 1940 and 1948 He concluded that among all adults over 20 years, peptic ulcer will at some future time develop in about 18 % of the men and in about 6 % of the women In agreement with this, Watkinson (24) who in 1960 published a thoroughly analysed pathological study found that the disease was present in about 14 % of all men and in 8 % of all women

In this century, several attempts have been made to throw light on the prognosis in cases of peptic ulcer which have primarily been given medical treatment Among the earliest of these, two extensive Scandinavian follow up studies deserve special mention, viz those presented by Nielsen (22) in 1919 and by Mattsson (20) in 1931

More recent follow up studies on the clinical course of ulcer disease have been reported by Natvig et al (21) and Qvigstad and Romcke (23) Krarup (14), Flood (6) and Malmros and Hiertonn (18) These studies have the following features in common average observation periods of 5—10 years a high follow up percentage and a reliable diagnostic technique based on barium meal examinations In 1963 Krause (15) published an extensive follow up study on peptic ulcer His series comprised 665 patients who had been given medical treatment including 318 who were subsequently subjected to surgical measures Krause s study differs from those mentioned above in two ways the observation periods

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[1] Present address Sotoften 12 Egå Denmark

TABLE I Survey of ulcer patients on the first admission to hospital in 1936—1945 The discharged patients are distributed according to age, radiographical findings and presence or absence of manifest bleeding

Age (yrs) Type of ulcer	Bleeding	10—19	20—29	30—39	40—49	50—59	60—69	70—79	Total	
Gastric	Present	—	—	—	3	2	2	—	7	
	Absent	2	6	7	10	16	7	3	51	58
Duodenal	Present	1	4	15	11	8	5	2	46	
	Absent	14	38	40	53	36	23	1	205	251
Pyloric	Present	—	—	—	1	1	2	—	4	
	Absent	—	—	2	4	5	1	1	13	17
Gastric and duodenal	Present	1	—	—	—	—	—	—	1	
	Absent	—	3	3	7	2	4	1	20	21
							Total			347

TABLE II Sex ratio of the patients on the first admission in 1936—1945

Sex	Gastric ulcer	Duodenal ulcer
M F	38 20=1 9 1	190 61=3 1 1

are from 25 to 35 years, and the series has been subjected to a careful statistical analysis

The purpose of the study reported here was to shed light on the long term prognosis of medically treated cases of the ulcer disease, with special reference to an analysis of the factors which are of prognostic significance

Material and methods

During the period from 1936 to 1945 inclusive a total of 371 patients with confirmed ulcers were admitted to the Department of Medicine Aarhus Amtssygehus

However 13 patients died during the first hospital stay, including 10 in whom death was due to haematemesis and/or melaena Eleven patients were subjected to gastric operation all because of pyloric stenosis referable to the ulcer disease

A total of 347 patients (table I) was discharged after medical treatment, which consisted of a special restricted diet for 4—6 weeks bed rest and if necessary administration of antacids These patients were followed up and a detailed analysis is reported below

First admission during the period 1936—1945

Manifest bleeding occurred in 58 or 17 % of the 347 patients (table I)

The age distribution shows that duodenal ulcer accumulated particularly in the 2nd to 4th decades of life while gastric ulcer mainly occurred in the 4th to 6th decades The difference is significant ($p < 0.01$)

The sex distribution shows a male preponderance especially among patients with duodenal ulcer The sex ratio in duodenal and gastric ulcers did not differ significantly (table II)

Types of ulcer It appears from table III that duodenal ulcer was more frequent than gastric ulcer especially in men, but the

TABLE III Ratio of duodenal to gastric ulcer on the first admission in 1936—1945

Type of ulcer	Men	Women	Total
Duodenal to gastric	190 : 38 = 5.0 : 1	61 : 20 = 3.1 : 1	251 : 58 = 4.3 : 1

TABLE IV Duration of symptoms before the first admission in 1936—1945

	Gastric ulcer		Duodenal ulcer	
Duration of symptoms (yrs)	No	%	No	%
$<1/2$	13	22	30	12
$1/2$—2	6	10	38	15
2—5	9	16	40	16
>5	30	52	143	57
Total	58	100	251	100

TABLE V Gastric acid secretion as determined by Ewald's test meal in 284 patients on the first admission in 1936—1945

	Duodenal ulcer		Gastric ulcer	
Kemp's index[1]	No	%	No	%
>100	123	53	18	35
<100	109	47	34	65
Total	232	100	52	100

[1] Expressed in terms of ml of 1/10 N HCl in 1 hour

difference between the two sexes is not significant on that point

Duration of symptoms In both types of ulcer symptoms had been present for more than 5 years in more than one half of the cases i e the series consists mainly of chronic cases (table IV)

Gastric acid secretion was studied by Ewald's test meal with aspiration after 1 hour From table V it appears that the acid secretion was significantly higher in duodenal than in gastric ulcers ($p < 0.02$)

On the basis of Ewald's test meal Kemp's index (13) was calculated This index is an expression of the total gastric acid secretion in terms of millilitres of 1/10 N HCl in 1 hour Kemp fixed the limit between normal gastric acid secretion and digestively increased acid secretion at 100 ml 1/10 N HCl in 1 hour

Follow up examination

The patients were followed up in 1963 after observation periods varying from 17 to 27 years Information was obtained of 99 % of the patients (table VI)

TABLE VI Follow up examination in 1963 The clinical course of the ulcer disease

	Gastric ulcer						Duodenal ulcer					
	♂		♀		Total		♂		♀		Total	
Clinical course	No	%	No	%	No	%	No	%	No	%	No	%
Favourable	9	24	10	50	19	33	49	26	20	33	69	28
Less favourable	10	26	4	20	14	24	26	14	13	22	39	16
Serious	19	50	6	30	25	43	114	60	27	45	141	56
Total followed up	38	100	20	100	58	100	189	100	60	100	249	100
Not followed up	—		—		—		1		1		2	
Total	38		20		58		190		61		251	

Henceforth, no specific mention is made of cases of pyloric ulcer or of concurrent gastric and duodenal ulcers as these groups were too small for statistical analysis

All patients concerned were followed to death or operation while patients who were alive but had not undergone gastric operation were requested to return to the hospital for an interview and barium meal examination

As regards the patients who had died before the follow up examination information as to the course of the ulcer disease and the cause of death was obtained through the patients own doctors and by means of death certificates

The hospital records for all patients who had been admitted to hospital for medical or surgical treatment of the ulcer disease during the observation period were reviewed

Clinical course According to the clinical *course of the ulcer disease the patients were* divided into three prognostic groups at the follow up examination

Group A Favourable course

Complete freedom from symptoms or only slight dyspepsia not requiring any treatment throughout the observation period

Group B Less favourable course

One re admission and/or one manifest bleeding and/or recurrence of ulcer associated with incapacity for work

Group C Serious course

Two or more re admissions and/or manifest bleedings or gastric operation, or death from ulcer disease

In order to study if certain factors determined on the first admission in the period 1936—1945 were related to the clinical course of the ulcer disease, the three groups just mentioned were compared with regard to the following alternative variables

Sex	Man	Woman
Gastric acid secretion	Above	< 100 ml 1/10 N HCl in 1 hour
Duration of symptoms	Less than	> 2 years
Age	Over	< 40 years
Haematemesis/ melaena	Yes	No

Statistically the case material was subjected to variance analyses and chi square tests

Results

Clinical course

It appears from table VI that duodenal ulcers ran a more serious course than gastric ulcers, but the differences were

TABLE VII Follow up examination in 1963 Frequency of gastric operation during the observation period

	Gastric ulcer		Duodenal ulcer	
	No	%	No	%
Operation	13	22	98	39
No operation	45	78	151	61
Total followed up	58	100	249	100

not significant In both types of ulcer, the clinical course was serious in about one half of the patients and favourable *in one third while the remaining cases* occupied an intermediary position

Complications

Perforation During the observation period, this complication occurred in 14 patients with duodenal ucler (5 6 %), whereas it was not seen in any of the gastric ulcer patients

Haematemesis/melaena occurred in 12 patients with gastric ulcer (21 %) and in 63 with duodenal ulcer (25 %) The difference is not significant

It should be noted that about 40 % of the patients in whom bleeding developed during the observation period also had haematemesis/melaena on the first admission

Gastric operation

During the observation period, gastric operation was performed in 39 % of the patients with duodenal ulcer as against only *in 22 % of the gastric ulcer patients* (table VII) The difference is significant ($p < 0.02$)

The indications for operation were in more than three quarters of the cases persistent symptoms which caused incapacity for work in spite of medical treatment In the remaining cases operation was performed because of pyloric stenosis haemorrhage or suspected malignancy, in that order of frequency

The operative mortality was about 8 %

Methods of operation Gastric operation was performed in 111 patients gastro-entero-anastomosis (which was very common up to the late 1940 s) in 22 % and gastric resection (usually by the method of Polya) in the remaining 78 %

Which factors are of prognostic significance?

Sex It is seen from table VI that men have a poorer prognosis than women For gastric and duodenal ulcers combined the difference between the two sexes is significant ($p < 0.05$)

Duration of symptoms The clinical course during the observation period was more serious in patients who had had symptoms for more than 2 years before the first admission than in those with a shorter duration of symptoms (table VIII) However the difference is significant only for duodenal ulcers ($p < 0.05$)

TABLE VIII Follow up examination in 1963 The clinical course related to the duration of symptoms before the first admission to hospital in 1936—1945

	Gastric ulcer				Duodenal ulcer			
	Duration of symptoms				Duration of symptoms			
	< 2 years		> 2 years		< 2 years		> 2 years	
Clinical course	No	%	No	%	No	%	No	%
Favourable	9	47	10	25	23	34	46	25
Less favourable	2	11	12	31	15	22	24	13
Serious	8	42	17	44	29	44	112	62
Total followed up	19	100	39	100	67	100	182	100

TABLE IX Follow up examination in 1963 The prognostic significance of gastric acid secretion as determined on the first admission to hospital in 1936—1945

	Gastric ulcer				Duodenal ulcer			
	Kemp s index[1]				Kemp s index			
	< 100		> 100		< 100		> 100	
Clinical course	No	%	No	%	No	%	No	%
Favourable	15	44	4	22	32	30	26	21
Less favourable	9	27	2	11	21	19	15	12
Serious	10	29	12	67	55	51	81	67
Total	34	100	18	100	108	100	122	100

[1] Kemp s index is expressed in terms of ml of 1/10 N HCl in 1 hour

Gastric acid secretion Table IX shows that patients with a high gastric acid secretion (i e above 100 ml 1/10 N HCl *in 1 hour as measured by the method* of Ewald) on the first admission had a poorer prognosis than those with a lower secretory capacity The difference is significant (p < 0 01)

Age at the first admission seems to be without any appreciable prognostic significance

Haematemesis/melaena It appears from table X that the prognosis is independent of the presence or absence of this *complication on the first admission* of the ulcer patients There is no significant difference between the two groups (p > 0 1)

The fate of ulcers

Table XI shows the changes which occurred during the observation period

TABLE X Follow up examination in 1963 The prognostic significance of haematemesis/melaena on the first admission to hospital in 1936—1945

Clinical course	Non bleeding ulcers No	%	Bleeding ulcers No	%
Favourable	86	30	14	25
Less favourable	50	17	8	14
Serious	152	53	34	61
Total followed up	288	100	56	100
Not followed up	1		2	
Total	289		58	
			Grand total	347

TABLE XI Follow up examination in 1963 Changes in the appearance of the ulcers in patients who had gastric or duodenal ulcer on first admission to hospital in 1936—1945

	On first admission in 1936—1945			
	Gastric ulcer		Duodenal ulcer	
At follow up in 1963	No	%	No	%
Gastric ulcer	23	40	6	2.5
Pyloric ulcer	2	3	32	13
Duodenal ulcer	3	5	119	47
Gastric and duodenal ulcer	2	3	10	4
No ulcer	18	31	55	22
Gastric cancer	1	2	1	0.5
No definite information	9	16	28	11
Total	58	100	251	100

in the appearance of the ulcers which were diagnosed on the first admission in the period 1936—1945

It must however be noted that the diagnostic methods used differed The aids used in the ulcer diagnosis during the observation period and at the follow up examination were radiography, operation or autopsy, while the diagnoses made on the first admission were exclusively based on barium meal examination

It is seen from table XI that about one quarter of the duodenal and about one third of the gastric ulcers healed without leaving radiographical sequelae It is also seen that *malignant tumours* were very rare both in gastric and

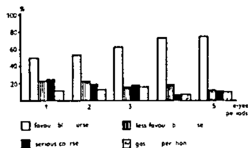


Fig 1

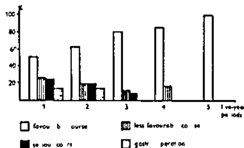


Fig 2

Figs 1 and 2 Clinical course of duodenal and gastric ulcer resp shown for individual 5 year periods The distribution of the patients is expressed as percentages of the total number of patients who were alive and had not undergone gastric operation For further explanation see text

duodenal ulcer patients In none of the cases could it be demonstrated that it was the ulcer which had developed into gastric cancer

The last group in table XI, of which no definite information is available, consists of patients who died during the observation period without having been subjected to diagnostic stomach examination Of these patients it is known that their gastric disease ran a quiet course and did not cause renewed admission to hospital None of these patients seem to have suffered from gastric cancer

Incidentally, the follow up examination revealed a certain discrepancy between the results of the barium meal examinations and the clinical symptoms About one quarter of the patients in whom peptic ulcer was demonstrated by radiography were free of symptoms, while, conversely, two thirds of the patients in whom radiography failed to reveal any ulcer still suffered from dyspepsia

Mental stress and ulcer

At the follow up examination it appeared that mental factors played an important part in the development, intensity and periodicity of the ulcer dyspepsia Thus, increased discomfort developed when the patient assumed work of greater responsibility, whereas in patients who went from leading to more subordinate positions, or from piece work to time work, the ulcer distress lessened Mental stress may, of course, also be due to other factors, such as marital or financial difficulties

Owing to the complex nature of this problem, the patients were merely asked if they found that there was any relationship between factors of mental stress and the ulcer symptoms The answers given by the patients showed that in two thirds of the cases there was a close relationship between mental stress and ulcer dyspepsia

Intensity of the ulcer disease during the observation period

In order to gain an impression of the importance of the length of the observation period in the assessment of the results of medical treatment of peptic ulcer, and to obtain results which may

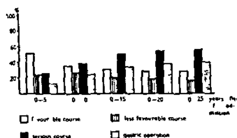


Fig 3

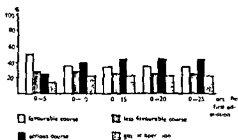


Fig 4

Figs 3 and 4 Cumulative evaluation of the clinical course of duodenal and gastric ulcer resp at 5 year intervals For further explanation see text

be compared with those reported by other authors, the series was also analysed for consecutive 5 year periods

Such an analysis may be made (a) by separate assessment of each 5 year period or (b) by a cumulative assessment in which the results for the entire preceding observation time is analysed at the end of each 5 year period Both methods give an impression of the ulcer disease as a function of the observation period

a Separate assessment of each 5 year period In figs 1 and 2, the intensity of the ulcer disease is plotted against the observation period for patients with duodenal and gastric ulcer, respectively On the abscissae of the figures the observation time is divided into 5 year periods At the end of each 5 year period the distribution of the patients in the three prognostic groups (i e favourable course less favourable course, and serious course) is calculated This distribution is expressed as percentages of the total number of patients who were alive and had not undergone gastric operation at the beginning of the 5 year period concerned In this way, the cumulative effect of patients who had died or been operated on in previous 5 year periods is eliminated The frequency of operations is recorded in the same way

It is seen that assessed by this method the intensity of the ulcer disease is definitely decreasing with the length of the observation period

b Cumulative assessment of the 5 year periods Figs 3 and 4 show a cumulative representation of the clinical course during the observation period in patients with duodenal and gastric ulcer respectively The records were made as follows

If, at some time a patient was assessed to belong to group C i e serious course he remained there for the rest of the observation period If a patient died of causes other than peptic ulcer he remained in the group in which he had finally been placed while alive In the course of the observation period patients might be moved down from favourable course to less favourable course and further to serious course but never the opposite way

It appears that assessed in this way the intensity of the ulcer disease also decreases with the length of the observa

tion period Balance is attained after the lapse of 15—20 years, and only after that period can the final assessment be made

Discussion

Only rough comparisons are possible between follow up studies of ulcer patients, because the selection of the patients and the criteria used in the classification vary to some extent from study to study, even though, in principle, attempts are made to tackle the prob lems in the same way

The composition of the series consider-ed here is similar to that found in other Scandinavian follow up studies of ulcer patients from the same period (14, 15, 18, 21, 23), i e all the studies showed a preponderance of male patients and of cases of duodenal ulcer There is also agreement with regard to the clinical course of the ulcer disease A favourable course can be expected in 15—30 % of the cases, with the best results in gastric ulcer and the poorest in duodenal ulcer However the difference between these two ulcer groups is small, it is clearly significant only in the series studied by Krause (15)

Among the aforementioned investiga tors only Krarup (14) found that ulcers associated with manifest bleeding had a better prognosis than non bleeding ulcers The explanation of this finding probably is that in his group of bleeding ulcers Krarup included some cases with haematemesis/ melaena in which there was no radio-graphically demonstrable ulcer, and in which the clinical course was better than in the cases with manifest bleeding associated with radiographically de-monstrable ulcer

The present study and those just mentioned agree that long duration of symptoms before the first admission to hospital is indicative of a poor prognosis

As distinct from the results reported by Krarup and Krause, the present study shows that sex is a significant prognostic factor, the outlook is poorer in men than in women On the other hand, all three studies agree that age at the first admission is of no prognostic significance

The relationship of gastric acid se cretion to the prognosis is an interesting problem which has not previously been sufficiently investigated The results of the analysis reported here suggest that a high acid production carries a poor prognosis However, the studies of gastric acid secretion were here based on Ewald's test meal, a method of investigation which has during recent years been abandoned in favour of the augmented histamine test introduced by Kay (12) in 1953, the latter is more accurate and gives reproducible values in the individual patient Nevertheless, it must be emphasised that the deter minations of gastric acid secretion per formed by means of Ewald's test meal are to a certain extent reproducible, as shown by Bennett and Ryle (2) in 1921 In 1960, Marks and Shay (19) studied 114 patients both by Ewald's test meal and by the augmented his tamine test They found that this group of patients as a whole showed a signifi cant correlation between the two tests but at the same time they emphasised

that the two tests showed wide variations in the individual patient

According to Garland (7) and Etter et al (5), barium meal examination gives rise to misdiagnosis in 10—30 %, whereas Hornnes and Kinsey (8) found erroneous diagnoses in 2—3 % of the cases The radiographic diagnoses in the present series were made by the same chief radiologist, both on the first admission to hospital and at the follow-up examination This should reduce the possibilities of misdiagnosis to a minimum In this connexion it is of interest to mention that only in two out of 76 patients who were subjected to gastric operation in our hospital did the operative findings differ from the interpretation of the pre-operative barium meal radiographs

At the follow up examination it was observed that a large number of both gastric and duodenal ulcers showed radiographical healing and that the clinical symptoms were not always in conformity with the radiographical findings These observations are both in agreement with those reported by Natvig et al (21)

A correlation between mental stress and ulcer dyspepsia is suggested by the present study The problem has been investigated in detail by Hojer Pedersen (9) who on the basis of a series of 51 ulcer patients and a control group concluded that sufferers from peptic ulcer are apt to transform an emotional conflict into dysfunction If the conflict cannot be relieved at a psychological or psychomotor level the only possibility left is a vegetative-endocrine discharge

Judging from the present investigation, no relationship seems to exist between peptic ulcer and gastric cancer This is in agreement with the findings of Krarup (14), Malmros and Hiertonn (18) and Krause (15) The basic problem involved in this discussion, which has sometimes been fairly ardent, is perhaps rather to be sought in the initial differential diagnosis between cancer and peptic ulcer

In the series considered here, the intensity of the ulcer disease was greatest during the first few years after diagnosis and then decreased, but only after the lapse of 15—20 years did it adjust itself on a roughly constant level In a follow up examination of ulcer patients an observation period of at least 15—20 years must therefore be required in order to determine the prognosis of the disease

A comparison of figs 1 and 2 with figs 3 and 4 clearly shows that the impression obtained of the clinical course of the ulcer disease depends on the methods used in recording and analysis The results of follow up studies are most commonly analysed according to the cumulative principle This depicts peptic ulcer as a progressive disease giving poorer results with longer observation periods When this principle is used sufficient regard is not paid to the long symptom free intervals which the patients may have between the recurrences during the observation period In his follow up study of 233 patients with duodenal ulcer after an observation period of 7 years, Flood (6) threw light on the problem by calculating the total number of years

which represented periods with and without symptoms He found that 63 % of the observation years were symptom free In the present study, the problem was tackled by analysing the results of individual 5 year periods of the observation time The results were roughly identical with those reported by Krause (15) and are suggestive of a more favourable course of the ulcer disease than is obtained by the cumulative method

It is thus difficult to arrive at a correct evaluation of the clinical course of peptic ulcer under medical treatment, but the cumulative method undoubtedly gives a too pessimistic picture of the prognosis

It is even more difficult to compare the results of medical versus surgical treatment of the ulcer disease, because the criteria used in the selection of the two types of patients differ Surgical treatment is usually employed in cases of recurrence associated with incapacity for work in spite of medical treatment Follow up studies of patients treated with gastric resection (3 15 16) show that satisfactory results are obtained in 80—85 % of the cases

However these percentages refer to short term results Krause emphasised that anaemia subsequently developed in one third of the patients subjected to gastric resection

More recent investigations into the late complications of gastric resection were published by Jespersen (10) Juul Jensen (11) and Crooks et al (4) It appears that anaemia malabsorption myopathy and bone disease subsequently develop in an appreciable number of the patients In 1965 it was pointed out in an Editorial in the Lancet (17) that the problem is serious, and that adequate long term follow up studies of surgically treated ulcer patients are not available

The ideal would be that it would be possible beforehand to single out the ulcer patients who ought to be subjected to gastric resection, i e those in whom medical treatment offers a poor prognosis At present, this cannot be done in the individual cases, but the investigation reported here shows that, in a group of patients, it may be said that long duration of symptoms, male sex and high gastric acid secretion are separately indicative of a poor prognosis Future studies of cases in which the augmented histamine test has been applied may reveal if there is a significant correlation between the accurately determined gastric acid secretion and the prognosis for the individual patient

Summary

A follow up study of 371 patients with peptic ulcer who were treated conservatively during the period 1936—1945 is reported

During the first hospital stay, 13 patients died, including 10 in whom haematemesis/melaena occurred, and gastric operation was performed in 11 cases

A total of 347 patients was discharged from hospital Of these, 38 had pyloric ulcer or concurrent gastric and duodenal ulcers

Information was obtained of 99 % of the remaining 309 patients (58 with

gastric and 251 with duodenal ulcer), and the survivors were subjected to a clinical and radiological follow up examination after observation periods of 17—27 years

The results of the follow up study were as follows

1 A favourable clinical course was seen in gastric and duodenal ulcers in 33 and 21 % respectively

2 Perforation occurred in 5 6 % of the patients with duodenal ulcer, but was not encountered in any of those with gastric ulcer

3 Haematemesis/melaena occurred in about one quarter of all the ulcer cases

4 Gastric operation was performed at some time during the observation period in 39 % of the cases with duodenal ulcer and in 22 % of those with gastric ulcer

5 The prognosis was poorer in men than in women

6 Long duration of symptoms before the first admission to hospital indicates a poorer prognosis

7 High gastric acid secretion on the first admission suggests a poorer prognosis

8 The clinical course of bleeding and non bleeding ulcers did not differ

9 From one quarter to one third of the ulcers healed without leaving radiographical sequelae

10 The radiographical follow up revealed a fairly wide divergence between the occurrence of dyspepsia and the demonstration of ulcer

11 In two thirds of the patients there was a close relationship between dyspepsia and mental stress

12 The intensity of the ulcer disease decreased during the observation period and did not become stationary until after the lapse of 15—20 years

13 No relationship was revealed between peptic ulcer and gastric cancer

Various methods of recording and analysis and conservative versus surgical treatment are discussed

Acknowledgement

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From Medical Department VIII (Head A Jervell M D) and the Central Laboratory (Head S L Sveinsson M D) Ullevål Sykehus Oslo, Norway

Liver Cell Affection in Myocardial Infarction

Revealed by Elevated Serum Ornithine Carbamoyl Transferase

By

JON DALE and INGVAR RUNDE

Determinations of serum glutamic oxala cetic transaminase SGOT, is of great importance in confirming the diagnosis of myocardial infarction (2, 7, 8, 18) Elevated SGOT-values are described in 96,9 to 99 per cent of the patients during the acute phase of the disease (1 4) However, GOT is not specific for the myocardium (18, 19), and SGOT is found elevated in many pathological conditions Since the concentrations of the various intracellular enzymes differ from organ to organ, determination of the serum activities of two or more enzyme may be valuable in the organ diagnosis Therefore the non specificity of GOT for the heart may be overcome by simultaneous determination of other serum enzymes The relation between SGOT and serum glutamic pyruvic transaminase, SGPT, has often been used in attemps to differentiate between heart and liver affection Elevation of SGPT is found in several diseases of the liver, and sometimes also in myocardial infarction (5, 9, 10, 19) Increased SGPT-activity after infarctions may originate from the myocardium or from the liver because of circulatory disturbances with liver congestion

The purpose of our investigation has been to demonstrate possible liver cell affection caused by myocardial infarction To accomplish this we have determined serum ornithine carbamoyl transferase SOCT OCT is found in the liver, but only in very small amounts in other organs except the small intestine (13) Thus elevated SOCT has been used in the present investigation to demonstrate liver cell affection The activities of GOT GPT and OCT in heart and liver are shown in table I We have also sought to investigate the diagnostic value of SGPT and the SGOT/SGPT-ratio by relating them to the corresponding SOCT values

Material

The material consists of 40 patients with acute myocardial infarction The diagnosis was based on common criteria (2) All pa

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TABLE III The SGOT/SGPT ratio in infarctions with SGPT elevation Group A and group B (table II) are compared as to the mean, calculated for each day and for maximum enzyme values in the patients The standard deviation (SD) and the t test on the differences are listed

SGOT/SGPT	Group A		Group B		t test
	Mean	SD	Mean	SD	
1st day	1 94	0 95	3 12	2 65	P = .20
2nd day	4 48	3 29	5 62	3 21	P > 50
3rd day	3 53	2 66	4 84	2 38	P > 20
Max values	3 59	2 63	5 59	2 51	P > 05

(group B) The differences between the ratios are calculated for each of the three days and also for the maximum enzyme values found in each patient (table III) However, the differences between the groups are not statistically significant

In 14 patients SGOT alone was elevated (group C) The maximum SGOT values in these patients were considerably lower than in cases giving pathological values of one or both of the two other enzymes determined

SGPT remained within normal limits in 7 patients with elevation of SOCT (group D) However, in this group the rise in SOCT values was very moderate with a highest value of 0 33 μ moles NH_3 slightly above the upper normal limit This group comprises one patient with lung edema 4 with roentgenologically enlarged hearts, one with congestion of the lungs and enlarged liver and finally one patient with a probable myocardial infarction 3—4 days before the definite one

Clinical manifestations of circulatory disturbances during the acute stage of the infarctions were found in 12 patients, of whom 7 had elevated SOCT-values Circulatory shock did not occur in any patient

On roentgenological examination of the patients who survived, pathological heart volumes were found in 60 % of the cases with demonstrated liver affection, while the hearts were enlarged in 23 % of the patients with normal SOCT

Discussion

Affection of the liver is found in as many as 45 per cent of our patients with myocardial infarction, demonstrated by the enzyme SOCT However the SOCT-elevation was moderate in most of the cases, exceeding 0,50 μ moles NH_3 in only three of them The SGOT values are not found higher in these patients than in those without liver affection The size of the infarction seems to be not the only factor determining whether or not a myocardial infarction leads to circulatory disturbances with liver cell injury One may assume that the condition of the heart and circulatory system

prior to the actual infarction is equally important This is supported by the fact that pathologically enlarged hearts were found in most of the patients with elevated SOCT values

In myocardial infarction SGPT, as well as SGOT, may be derived from the heart and the liver With regard to the infarctions causing pathological SGPT values, it may seem strange that SGOT is found highest in the group without affection of the liver (group B) However, the activity of GPT per gram of heart muscle is only 5 % of that in the liver A myocardial infarction without liver cell affection must therefore be of a certain magnitude to release so much GPT into the blood stream that pathological SGPT values can be found This accords with the suggestion of Wroblewski and LaDue (19) that only infarctions with SGOT higher than 150—200 units will cause SGPT elevation

A further point concerning infarctions with pathological SGPT values is that the mean SGOT/SGPT ratio is highest in the group without liver cell affection, as might be expected There is a considerable variation from patient to patient, and the difference in SGOT/SGPT ratio between the groups are not statistically significant However the tendency is so marked that a larger material possibly would reveal significant differences In cases where the liver is affected SGPT is derived partly from the liver and partly from the heart This is probably the explanation of the SGPT values being found higher in group A than in group B (table II) In an isolated case of myocardial infarction, however, the SGOT/SGPT ratio is a poor sign of liver cell affection, unless SGPT reaches particularly high values

Group C includes infarctions with so limited necrosis that SGPT remains normal, and liver cell affection is not demonstrated in these patients As expected the SGOT elevation in this group is moderate

In the patients who belong to group D, only slight liver cell affection was found, with SOCT values just above the upper normal limit and SGPT normal The enzyme values found in these patients seem to indicate that SOCT is more sensitive for demonstration of liver cell affection than is SGPT This suggestion is supported by the results from an investigation concerning tachycardia without simultaneous myocardial infarction where SOCT more frequently reached pathological values than did SGPT (16) In all infarctions that caused SOCT to exceed 0,33 μ moles NH_3 the SGPT was found elevated as well

Summary

In 40 patients with myocardial infarction 3 serum enzymes SGOT SGPT and SOCT, were determined daily for 3 days Pathological values of the SOCT serum ornithine carbamoyl transferase are found only in conditions with liver cell damage In as many as 45 per cent of our cases elevated SOCT values demonstrated liver cell affection which however was moderate in most of the patients The myocardial necrosis of infarctions leading to affection of the

liver was not particularly extensive, since the mean SGOT values were not higher than in the other infarctions Whether the liver will be affected therefore seems to depend not only on the size of the infarction, but also on other factors, such as the condition of the heart and circulatory system prior to the infarction

The diagnostic value of SGPT determination in myocardial infarction is considered The SGPT was elevated in 19 cases, 11 with and 8 without affection of the liver cells The mean of the maximum SGPT values was somewhat higher where liver affection was demonstrated, but the variation was great and the difference between the 2 groups was not significant For the purpose of revealing affection of the liver in myocardial infarction determination of the SGPT therefore is of limited value, and the SOCT should be preferred

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From the Second University Clinic of Internal Medicine (Head K Lundbæk M D)
Kommunehospitalet Aarhus Denmark

The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method

VI Insulin like Activity in Serum and Serum protein Fractions from Normal Persons and Patients with Diabetes Mellitus

By

J Lyngsoe and K Lundbæk

Studies published during recent years seem to indicate that serum contains several different types of insulin (1 2, 3, 6, 7, 11, 18, 19, 22, 23 24 26 27) Our own results suggest that serum contains a type of insulin which is inactive on rat epididymal fat in vitro and that on electrophoresis this type of insulin is found localized to the albumin α_1 globulin (the A fraction) as well as to the β-γ globulin (the B fraction) (18 19) More recent investigations seem to suggest that insulin which is localized to the A fraction and B fractions is produced in the pancreas (11 20), and that after administration of glucose the insulin localized to the A fraction is retained in the peripheral tissue (20) This state of affairs suggests that insulin localized to the A and B fractions has a certain significance for the normal regulation of the blood sugar level notwithstanding the fact that these insulin types are without biological activity in vitro A study was therefore made of the occurrence of ILA in the A and B fractions in the serum from a small group of patients with diabetes mellitus, using a special technique whereby the determination was made simultaneously on serum from a normal subject and on serum from a patient with diabetes mellitus in order to increase the comparability of the results

Furthermore, both in the normal subjects as well as in the diabetic patients, determinations were made of the suppressible and non suppressible serum insulin like activity in those sera used for recording the ILA in the A and B fractions

Submitted for publication April 12 1966

Material and methods

The normal subjects were patients admitted to a medical department with diseases un

TABLE I Normal persons investigated in the fasting state and 10 minutes after intravenous glucose

Pat no	Assay no	Lambda	Serum glucose (mg %)	Suppressible SILA (μU/ml)		Non suppressible SILA (μU/ml)	
				I	II	I	II
16	1 022	0 15	100–214	–5	75	95	90
17	1 033	0 21	89–216	2	123	66	102
20	1 040	0 34	92–220	12	71	77	59
26	1 052	0 32	81–226	122	142	113	108
33	1 061	0 29	85–190	60	201	50	39
48	1 092	0 31	84–204	22	53	13	9
50	1 101	0 24	89 187	6	42	56	32
52	1 103	0 18	95–210	64	160	68	140
54	1 109	0 34	100–262	45	123	210	132
60	1 117	0 34	90–197	95	440	180	130
62	1 124	0 29	83–225	52	243	118	87
58	1 115	0 29	103 276	73	90	47	30
66	1 148	0 21	90–241	28	75	67	120
68	1 144	0 27	92–182	93	243	77	72
71	1 155	0 40	77–175	0	33	93	60
73	1 160	0 34	71 185	34	141	0	17
75	1 170	0 29	8–189	78	304	62	46
77			95				
Mean value ± standard dev				45±40	151±108	82±52	75±43

accompanied by elevated blood sugar or glycosuria They had no symptoms of diabetes mellitus and an examination of the 24 hour urine with Clinistix showed no glycosuria An examination of the sera used for the determination of insulin like activity of serum (SILA) showed fasting serum glucose values of less than 105 mg%

The patients who were examined were all untreated recently diagnosed diabetics hospitalized in a medical department None of the patients were suffering from malignant diseases or icterus They were all afebrile at the time of examination The diabetic patients had been on a completely normal unrestricted diet prior to the investigation

Blood samples for determination of SILA were taken from an arm vein under slight stasis All the patients with the exception of patient no 7 were examined between 7 and 8 a m, after fasting and bed rest for 8–12 hours Patient no 7 was examined at 4 p m after fasting and bed rest for 4 hours After taking the fasting blood sample an intravenous injection of 50 ml 50 % glucose was given in the course of one minute Nine minutes after completing the injection a second venous puncture was performed This latter blood sampling lasted between 1 and 2 minutes and was designated examination 10 minutes after intravenous glucose administration

SILA was determined on fresh serum by a modification of the rat epididymal fat method 14) Serum for determination of SILA was prepared from venous blood as previously described (15) Undiluted serum was examined for SILA both with and without the addition of anti insulin An amount of 50 μl anti insulin was added per ml serum

administration

		ILA of the A fraction (μU/ml)		ILA of the B fraction (μU/ml)	
Assay no	Lambda	I	II	I	II
1 028	0 20	1 120	960	2 700	3 200
1 037	0 25	4 700	1 920	3 000	4 000
1 046	0 30	1 400	980	1 700	2 140
1 056	0 34	900	670	1 090	2 240
1 065	0 19	860	960	1 700	960
1 096	0 34	260	220	320	500
1 105	0 17	580	880	1 520	4 000
1 107	0 29	2 160	760	2 160	2 160
1 134	0 26	380	680	1,500	960
1 136	0 21	560	1 340	1 900	2 000
1 130	0 24	1 020	860	2 300	1 860
1 121	0 35	780		2 400	
1 150	0 21	5 800	4 800	5 000	4 500
1 146	0 23	3 600	3 000	5 700	3 000
1 157	0 12	1 280	1,280	3 000	1 900
1 163	0 20	1 540		1 680	
1 181	0 35	720	200	1 740	640
1 176	0 22	3 600		6 100	
		1 737±1 606	1 301±1 18э	2 528±1 567	2 271±1,253

The anti insulin was prepared as previously described (18)

The insulin like activity (ILA) was examined in serum protein fractions which were prepared by electrophoresis in poly vinylchloride blocks After the electrophoretic separation the fractions were dialyzed against running water dextran and Krebs Ringer bicarbonate buffer as described in a previous study (19) In some sera the ILA was examined in 8 fractions while in others the proteins were divided into 2 fractions only In the latter cases the A fraction contained albumin α_1 globulin and α_2 globulin while the B fraction contained α_2-globulin β globulin and γ globulin After terminating the dialysis the protein fractions were stored for 8—72 hours at 4 C

In the present investigation a normal subject and a diabetic patient were examined as a pair This meant that electrophoresis and dialysis were carried out in serum from one diabetic patient and one normal subject simultaneously both subjects belonging to the same age group and sex Likewise ILA in the dialyzed serum protein fractions from the paired subjects was examined on the same day The ratio between the ILA in the serum protein fractions from the diabetic patient and from the normal subject examined simultaneously was then calculated (D/N index)

Blood sugar determinations by Hagedorn Norman Jensen s method were made on the patients at 7 a m 1 p m and 5 p m respectively (12) Total CO_2 in the blood was determined by van Slyke s method (28) The glucose content of the urine was determined by a reduction method with potassium ferricyanide

TABLE I Normal persons investigated in the fasting state and 10 minutes after intravenous glucose

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26	1 052	0 32	81–226	122	142	113	108
33	1 061	0.29	85–190	60	201	50	39
48	1 092	0 31	84–204	22	53	13	9
50	1 101	0 24	89–187	6	42	56	32
52	1 103	0 18	95–210	64	160	68	140
54	1 109	0 34	100–262	45	123	210	132
60	1 117	0 34	90–197	95	440	180	130
62	1 124	0 29	83–225	52	243	118	87
58	1 115	0 29	103–276	73	90	47	30
66	1 148	0 21	90–241	28	75	67	120
68	1 144	0 27	92–182	93	243	77	72
71	1 155	0 40	77–175	0	33	93	60
73	1 160	0 34	71–185	34	141	0	17
75	1 170	0 29	78–189	78	304	62	46
77			95				
Mean value±standard dev				45±40	151±108	82±52	75±43

accompanied by elevated blood sugar or glycosuria They had no symptoms of diabetes mellitus and an examination of the 24 hour urine with Clinistix showed no glycosuria An examination of the sera used for the determination of insulin like activity of serum (SILA) showed fasting serum glucose values of less than 105 mg%

The patients who were examined were all untreated recently diagnosed diabetics hospitalized in a medical department None of the patients were suffering from malignant diseases or icterus They were all afebrile at the time of examination The diabetic patients had been on a completely normal unrestricted diet prior to the investigation

Blood samples for determination of SILA were taken from an arm vein under slight stasis All the patients with the exception of patient no 7 were examined between 7 and 8 a m after fasting and bed rest for 8—12 hours Patient no 7 was examined at 4 p m after fasting and bed rest for 4 hours After taking the fasting blood sample, an intravenous injection of 50 ml 50 % glucose was given in the course of one minute Nine minutes after completing the injection a second venous puncture was performed This latter blood sampling lasted between 1 and 2 minutes and was designated examination 10 minutes after intravenous glucose administration

SILA was determined on fresh serum by a modification of the rat epididymal fat method (14) Serum for determination of SILA was prepared from venous blood as previously described (15) Undiluted serum was examined for SILA both with and without the addition of anti insulin An amount of 50 μl anti insulin was added per ml serum

Urine after examination	24 hour urine on day of examination	Total CO_2 (mEq/l)
+L−G	62 g+L−G	
+L−G	133 g+L−G	
−L−G	45 g−L−G	
−L−G	5 g−L−G	
−L−G	54 g−L−G	
−L−G	46 g−L−G	
−L−G	27 g−L−G	
+L−G	97 g−L−G	
−L−G	83 g−L−G	
−L−G	19 g−L−G	
−L−G	8 g−L−G	
−L−G	15 g−L−G	
−L−G	31 g−L−G	
−L−G	4 g−L−G	
−L−G	78 g−L−G	
−L−G	68 g−L−G	
−L−G	280 g+L−G	24
+L+G	110 g+L+G	25
+L−G		20
−L−G	7 g−L−G	
+L+G	82 g+L+G	24
−L−G	17 g−L−G	
−L−G	17 g−L−G	

Patients with diabetes mellitus

The diabetic patients were classified into 3 groups according to clinical criteria on the lines of previous investigations (16, 17) Patients under 40 years of age on diagnosis of diabetes mellitus were designated *juvenile diabetics* Non obese patients who were over 40 years of age on diagnosis of diabetes were designated *older non obese diabetics* whereas the group *older obese diabetics* comprised obese patients in the same age group Obesity is defined as a body weight $\geq 115\%$ of an ideal weight, estimated on the basis of the Hafnia table of mean weights for a normal Danish population (15)

It appears that the group comprising *juvenile diabetics* shows mean fasting values for suppressible and non suppressible SILA higher than the corresponding values for normal subjects (table III), although the difference is not statistically significant ($p > 0.1$) Only one of these patients shows a considerable rise in immunological SILA after administration of glucose, and the mean values for both suppressible and non-suppressible SILA correspond to the fasting values

The group comprising *older non-obese diabetics* show mean values for suppressible and non suppressible SILA in agreement with the corresponding normal values No statistically significant rise in suppressible SILA can be recorded in these patients after administration of glucose ($p > 0.1$)

The mean fasting value for suppressible SILA in the group comprising *older obese diabetics* is 80 μU ml which is significantly higher than the corresponding normal value ($t = 2.120$ $0.05 > p > 0.02$) No statistically significant rise in suppressible SILA is found after administration of glucose ($p > 0.1$) In the same patient group the average fasting value for non suppressible SILA is found to be 133 μU ml which is significantly higher than the corresponding normal value ($t = 2.221$ $0.05 > p > 0.02$) A significant fall in non suppressible SILA ($t = 2.406$ $0.02 < p < 0.05$) is observed after glucose administration The mean value after glucose is higher than in the normal material but the

TABLE III Suppressible and non suppressible SILA in diabetic patients investigated in the fasting state and 10 minutes after intravenous glucose administration

Pat no	Assay no	Lambda	Serum glucose (mg %)	Suppressible SILA (μU/ml)		Non suppressible SILA (μU/ml)	
				I	II	I	II
Juvenile diabetics (age <40)							
15	1 021	0 21	240–377	142	54	163	51
51	1 102	0 22	290–410	10	18	118	110
72	1 159	0 35	166–342	60	80	165	85
74	1 169	0 27	198–310	100	207	88	108
76	1 171	0 40	320	61		16	
Mean value				75	90	110	86
Non-obese maturity onset diabetics (age 40)							
18	1 034	0 20	274–412	87	45	78	75
34	1 062	0 29	215–339	95	75	110	60
47	1 091	0 32	183–312	19	19	15	0
49	1 100	0 34	182–332	55	72	68	68
59	1 116	0 20	127–274	-55	130	325	160
61	1 123	0 27	179–342	96	235	132	110
Mean value				50	96	121	79
Obese maturity onset diabetics (age >40)							
19	1 039	0 25	253–335	93	63	122	52
25	1 051	0 33	147–235	155	233	140	97
53	1 108	0 31	245–368	108	65	228	160
57	1 114	0 31	250–367	48	0	72	85
63	1 127	0 25	195–309	85	163	220	152
65	1 147	0 19	160–265	73	125	112	140
67	1 143	0 19	245–333	20	150	140	110
70	1 154	0 31	200–327	57	9	33	15
Mean value				80	101	133	101

difference is not statistically significant ($p > 0 1$)

Figs 1—6 show the distribution of ILA in 8 electrophoretically separated serum protein fractions from 6 diabetic patients, viz 2 juvenile diabetics (figs 1—2), 2 older non-obese diabetics (figs 3—4), and 2 older obese diabetics (figs 5—6) The figures show that in these patients, as in normal subjects, the ILA maxima are localized to albumin α_1-globulin and to β-γ globulins In two patients no clear ILA maximum can be seen corresponding to albumin α_1-globulin, but no patient shows an ILA maximum which has an electrophoretic localization differing from that in normal subjects

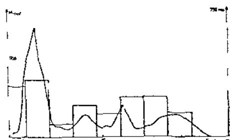


Fig 1 Patient no 72 Assay no 1164 lambda 0 29 This figure and the following ones show the proteinogram and the ILA of the protein fractions (columns)

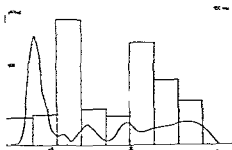


Fig 2 Patient no 79 Assay no 1005 lambda 0 31

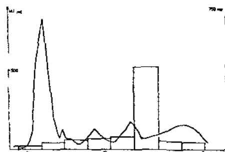


Fig 3 Patient no 78 Assay no 931 lambda 0 29

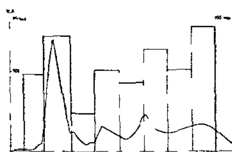


Fig 4 Patient no 81 Assay no 1162 lambda 0.29

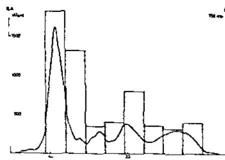


Fig 5 Patient no 70 Assay no 1158 lambda 0 12

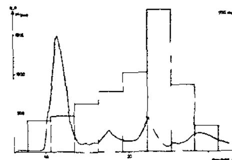


Fig 6 Patient no 80 Assay no 1161 lambda 0 27

Table IV shows the determinations of ILA in the two fractions designated the A fraction and the B fraction In the group of *juvenile diabetics* mean fasting values as well as mean values after glucose administration were found to be higher than the corresponding values in normal subjects All determinations on the A fractions from the 5 juvenile diabetics showed higher ILA values than

TABLE IV ILA of A and B fractions in diabetic patients investigated in the fasting state and 10 minutes after intravenous glucose

Pat no	Corresponding normal person	Assay no	Lambda	ILA of the A fraction I (μU/ml)		ILA of the A fraction II (μU/ml)		ILA of the B fraction I (μU/ml)		ILA of the B fraction II (μU/ml)	
Juvenile diabetics (age <40)											
15	16	1 027	0 17	1,160	(1 04)	1 900	(1 98)	2 560	(0 95)	4 800	(1 50)
51	52	1 106	0 18	3 700	(1 71)	3 160	(4 16)	3 700	(1 71)	3 500	(1 62)
72	73	1 163	0 20	1 640	(1 07)			3 000	(1 79)		
74	75	1 180	0 18	900	(1 25)	1 460	(7 30)	2 900	(1 67)	3 200	(5 00)
76	77	1 176	0 22	6 800	(1 8[illegible])			8 800	(1 45)		
Mean value				2 840		2 173		4 192		3 833	
Non obese maturity onset diabetics age 40)											
18	17	1 038	0 29	22 000	(4 68)	5 600	(2 92)	11 600	(3 87)	9 000	(2 25)
34	33	1 066	0 34	640	(0 74)	240	(0 25)	1 440	(0 85)	700	(0 73)
47	48	1 095	0 22	440	(1 69)	320	(1 45)	700	(2 19)	500	(1 00)
49	50	1 104	0 17	2 300	(3 97)	2 040	(2 32)	3 600	(2 37)	1 660	(0 42)
59	60	1 135	0 31	1 280	(2 28)	740	(0 55)	1 060	(0 56)	1 440	(0 72)
61	62	1 129	0 20	640	(0 63)	760	(0 88)	1 960	(0 85)	3 100	(1 67)
Mean value				4 550		1 617		3 393		2 733	
Mean value (pat no 18)				1 060		820		1 752		1 480	
Obese maturity onset diabetics (age 40)											
19	20	1 045	0 30	2 160	(1 54)	2 800	(2 86)	4 200	(2 47)	5 100	(2 38)
25	26	1 055	0 23	1 640	(1 82)	1 800	(2 69)	2 240	(2 06)	2 800	(1 25)
53	54	1 133	0 18	540	(1 42)	880	(1 29)	1 860	(1 24)	1 680	(1 75)
65	66	1 149	0 24	3 400	(0 59)	1 700	(0 35)	4 900	(0 98)	5 400	(1 20)
67	68	1 145	0 13	1 800	(0 50)	1 400	(0 47)	8 000	(1 40)	3 110	(1 04)
70	71	1 156	0 19	1 040	(0 81)	1 280	(1 00)	2 860	(0 95)	2 700	(1 42)
57	58	1 121	0 35	400	(0 51)			3 200	(1 14)		
63		1 131	0 32	1 020		820		4 200		4 900	
Mean value				1 500		1 526		3 933		3 670	

The figures in brackets indicate the D N index (see text)

those found in the normal subjects examined at the same time (D/N indices greater than 1) The mean values for ILA in the B fractions from juvenile diabetics were likewise higher than the values for normal subjects, and in 7 out of 8 determinations, D/N indices were found greater than 1 These investigations thus seem to suggest that juvenile diabetics have increased ILA values corresponding to both the A fraction and the B fraction

In the group comprising *older non obese diabetics*, the mean values found for ILA in the A and B fractions were higher than normal This is due to the fact that one patient (no 18) has considerably higher ILA values than those found in the remainder of the patients in this group When the 5 remaining patients are considered together mean ILA values in the A and B fractions are found to be a little lower than those in the normal material but as the corresponding D/N indices are evenly distributed above and below 1 it is hardly justifiable to regard these differences as significant

The determinations made in patient no 18 are of considerable interest All determinations of ILA in the A and B fractions from this patient showed exceptionally high values, and all D/N indices were correspondingly high This is in agreement with the findings in the juvenile diabetics, and the patient presented a clinical picture which is usual for this type of diabetes Without any signs of malignant disease or infection the patient was admitted to hospital with a very high blood sugar level and pronounced ketonuria and after a few days treatment with insulin was necessary because of a threatening acidosis After a few months the insulin treatment had to be tapered off to 8 units a day because of hypoglycaemic episodes

In the group consisting of *older obese diabetics* the mean values for ILA in the A fractions were of the same order of magnitude as in normal subjects In the B fractions on the other hand the mean ILA values were higher than in the normal material, and D/N indices in 11 out of 13 determinations were correspondingly greater than 1 ($p = 0.02$) The assumption seems justifiable therefore, that the ILA in the B fraction is increased in older obese diabetics

No definite change in ILA value following glucose administration could be demonstrated in the A or B fractions in any of the 3 groups of patients with diabetes mellitus

Discussion

In the present study an attempt has been made to elucidate the occurrence of different types of serum insulin in normal subjects and in patients with diabetes mellitus The investigation was carried out by determining non suppressible and suppressible SILA in peripheral venous serum as well as ILA in the electrophoretically separated A and B fractions The patient series examined was small but by making parallel determinations in a diabetic patient and in a normal subject the comparability was increased

In undiluted serum from normal subjects values for suppressible SILA were found which were of the same order of magnitude as those demonstrated in previous studies on diluted serum (5 9 21, 24) In agreement with the studies cited a rise in suppressible SILA was demonstrated following administration of glucose while non suppressible SILA was found to be unchanged

As in previous studies (18, 19) the determination of ILA in electrophoretically separated serum protein fractions

showed values that were markedly higher than those measured in unfractionated serum The present mean values for ILA in the A and B fractions from normal subjects are in complete agreement with the values determined by the same technique in a previously examined normal series (19) These findings demonstrated that corresponding to both albumin α_1 globulin and β γ globulin, an insulin like activity is present which has no effect on rat epididymal fat in untreated serum, but which is activated by electrophoresis and the following dialysis Previous investigations suggest that this phenomenon is based on an activation of insulin, present in untreated serum in an inactive, presumably protein bound form, both in albumin α_1 globulin and in β γ globulin (18) The present study demonstrated that after intravenous administration of glucose ILA in the A fraction was lower than in the fasting condition though the difference was not statistically significant An investigation in dogs gave similar results, and demonstrated further that soon after intravenous administration of glucose ILA in the A fraction was retained in peripheral tissue (20)

In the present study, an examination of the electrophoretic distribution of ILA in serum from *patients with diabetes mellitus* demonstrated a distribution corresponding to that found in normal subjects In *two patients, however, it was* not possible to demonstrate any ILA maximum corresponding to albumin-α_1 globulin In a study on the rat diaphragm method, Taylor (29) made a similar investigation, and found ILA in α_2 globulin in one of six patients with untreated diabetes mellitus and ketonuria, but he found no ILA in this fraction in normal subjects The other patients showed ILA in the albumin and β-γ globulin fractions, corresponding to the findings in normal subjects It is possible, therefore, that certain patients with diabetes mellitus may have an abnormal electrophoretic localization of the insulin like activity

Just as in other studies (8, 16, 17), the present investigations on patients with diabetes mellitus have shown that diabetic patients of various clinical types present different abnormalities with respect to their serum insulin Juvenile diabetics showed normal suppressible and non suppressible SILA, and ILA values in the A and B fractions which were higher than those found in normal subjects In the group of older non obese diabetics, normal values were found for suppressible and non suppressible SILA, as well as normal values for ILA in the A and B fractions In older obese diabetics elevated values were found for both suppressible and non suppressible SILA, the ILA in the A fraction was normal, while the ILA in the B fraction was elevated A feature common to all three groups of diabetics was that no rise in suppressible SILA could be demonstrated 10 minutes after intravenous administration of glucose, in contrast to what is observed in normal subjects *A fall in non suppressible* SILA after the administration of glucose was found only in the group of older obese diabetics

In agreement with previous investigations (4 9, 25) the present results obtained with *juvenile diabetics* likewise seem

to suggest that insulin in an immunologically active form is present in the peripheral venous blood of these patients, and that a rise in blood sugar does not increase the concentration Since normal pancreatic insulin production rises in step with rising blood sugar level, it is reasonable to assume that an increased concentration of insulin in the peripheral blood corresponds to an elevated blood sugar level The finding of normal values for suppressible SILA in juvenile *fasting* diabetics, despite an elevated blood sugar level suggests that these values are relatively reduced, presumably as a result of a reduced pancreatic secretion of the immunologically active type of insulin

On the other hand, the present study on ILA in the A and B fractions seems to suggest that these fractions are elevated in peripheral venous blood from *juvenile diabetics* More recent investigations are compatible with the assumption that the ILA in these two fractions is due to insulin which in untreated serum is inactive on rat epididymal fat in vitro and that these two insulin fractions are produced in the pancreas (11 18 20) The elevated values found for ILA in the A and B fractions in peripheral venous blood seem to support the assumption that this reflects an increased pancreatic production of the ILA measured in these fractions though the possibility of a reduced retention in the peripheral tissues and in the liver (20) cannot of course be excluded

As in previous studies (26) normal values for suppressible and non suppressible SILA were found in the group of *older non obese diabetics* and no rise in these values followed administration of glucose As was the case in the group of juvenile diabetics, this finding must be regarded as an expression of a decreased pancreatic secretion of immunologically active insulin Normal values for ILA corresponding to the A and B fractions were found in the older non-obese diabetics This suggests that the pancreatic production of these two types of ILA has not increased It is probable that these findings reflect an abnormal insulin production in the older non obese diabetics, and that the abnormality differs in kind from that found in the juvenile diabetics

Previous investigations on *untreated older obese diabetics* showed an elevated SILA which fell to normal values following dietary treatment (8, 17) The present study suggests that the increase in SILA is due to an increase in both suppressible and non suppressible SILA In contrast to this finding Samaan and Fraser (25) in older obese diabetics obtained an increase in non suppressible SILA but normal values for suppressible SILA Recent investigations by means of an immunological method, however, have supported the assumption that an increased quantity of insulin in an immunologically active form is found in the peripheral venous blood of these patients (13)

It is of considerable interest to notice that in contrast to the findings in normal subjects a fall in non suppressible SILA begins after intravenous administration of glucose in older obese diabetics

In the present study, older obese diabetics did not show a rise in suppr-

sible SILA, 10 minutes after intravenous glucose administration This finding is apparently in contrast to the finding in a previous investigation where it was possible to demonstrate a pronounced rise in SILA, 90 minutes after oral administration of glucose (16) Yalow and Berson (31), on the other hand, were able to demonstrate that after administration of glucose, a rise in immunologically determined insulin occurred more slowly in a group of older patients with "mild" diabetes than in normal subjects If the findings in older obese diabetics prove similar to the findings in Yalow and Berson's group, the conclusion may be drawn that previous and present investigations are compatible

In untreated older obese diabetics, the finding of an elevated level of both suppressible and non suppressible SILA suggests that where they are untreated, these patients have an increased pancreatic production of insulin in the immunologically active form Some time after oral administration of glucose these patients show a rise in both immunologically determined insulin and SILA, and during dietary treatment, a decrease in SILA in step with the blood sugar These observations justify the assumption that in such patients, the production of the immunologically active type of insulin is regulated in step with changes in the blood sugar level, just as in normal subjects In older obese diabetics under dietary treatment however, normal SILA values are found in spite of increased fasting blood sugar This may mean that the pancreatic production of immunologically active insulin is not increased in this condition In other words, this state of affairs suggests that there is an abnormal regulation of insulin production in older obese diabetics

The finding that untreated older obese diabetics have increased ILA in the B fraction, but normal ILA in the A fraction, is of considerable interest On the assumption that the insulin in both the A and the B fraction is produced in the pancreas, dependent on the blood sugar level, it seems justifiable to interpret increased ILA in the B fraction as an expression of increased pancreatic production of insulin with this electrophoretic localization Animal experiments have shown, however, that in the peripheral tissue there is a retention of ILA localized to the A fraction A normal level for this type of ILA in peripheral serum can therefore either be due to a normal pancreatic production with a normal retention in the peripheral tissue, or to an increased pancreatic production with an increased retention in the periphery The present investigations are thus compatible with the hypothesis that untreated older obese diabetics have an increased pancreatic production both of suppressible SILA and of ILA in the A and B fractions, but that the retention of ILA corresponding to the A fraction is increased in the peripheral tissue

Summary

In a series comprising normal subjects and patients with diabetes mellitus, investigations were made on non suppressible and suppressible SILA, as well as on ILA in two electrophoretically sepa

rated serum protein fractions the A fraction (albumin and α_1 globulin) and the B fraction (β and γ globulin) The studies on ILA in the electrophoretically separated protein fractions were performed on paired sera from a normal subject and a patient with diabetes mellitus simultaneously, with a view to increasing the comparability

On examining peripheral venous serum from normal subjects, a rise was found in the values for suppressible SILA 10 minutes after intravenous administration of glucose, while the values for non suppressible SILA were found unchanged The values for ILA in the A and B fractions were not found significantly lower after administration of glucose

In patients with diabetes mellitus, no change in suppressible SILA could be demonstrated following intravenous glucose administration A fall in non suppressible SILA could be demonstrated in older obese diabetics, whereas no significant change could be demonstrated after glucose administration in juvenile diabetics and in older non-obese diabetics

Normal values were found for suppressible and non uppressible SILA in a group consisting of juvenile patients with diabetes mellitus whereas values for ILA in the A and B fractions were higher than the values in normal subjects

A group comprising older non obese patients with diabetes mellitus showed normal values for suppressible and non suppressible SILA and normal values for ILA in the A and B fractions

A group consisting of older obese diabetics showed elevated fasting values for suppressible and non suppressible SILA as well as normal values for ILA in the A fraction and elevated values for ILA in the B fraction

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From the Cardiovascular Laboratory of Medical Department B (Head A Tybjærg Hansen), Rigshospitalet (University Hospital) Copenhagen Denmark

Right-heart Intracardiac Phonocardiography in Patients with Aortic Stenosis

By

ALF WENNEVOLD

Aortic systolic murmurs are transmitted to the right side of the heart and may thus be recorded with intracardiac phonocardiography during a right heart catheterization (1 4 7)

The relative intensity of the murmur in the different sites of the right heart seems to be of diagnostic help in the differentiation between the various types of aortic stenosis (3 7)

In this paper a more detailed analysis of the intensity of the murmur is made in a larger group of patients, and the diagnostic significance is re evaluated

Methods and material

Pressure and intracardiac phonocardiogram were recorded with the Allard Laurens micromanometer (6) During the catheterization the murmurs were followed on a loud speaker and registered on tape

This study comprises 22 patients (table I) Thirteen of the patients had valvular aortic stenosis one patient had bicuspid aortic valve without any stenosis three patients had fibrous subvalvular aortic stenosis two patients had typical severe subaortic muscular stenosis one patient had slight outflow tract stenosis one patient had subvalvular stenosis of unknown type and one patient had both a valvular and a subvalvular pressure gradient

The diagnosis was in 16 patients established at transseptal left heart catheterization with recording of the pressure gradient across the valvular or subvalvular area (table I) In two patients with valvular stenosis the diagnosis was confirmed at autopsy (cases no 7440 and 7506) Thoracic aortography demonstrated the stenosed valve in one patient (case no 7564) and established the diagnosis of bicuspid aortic valve in another (case no 7369) (7)

One patient with fibrous subvalvular stenosis (case no 7545) had the diagnosis confirmed at open heart operation In case no 7746 thoracic aortography demonstrated normal aortic valve

In the two patients with severe subaortic muscular stenosis (cases no 7573 and 7603) the characteristic pressure tracing was found in the outflow tract of the left ventricle In case no 6676 there was a gradual decrease of pressure across the outflow tract without any characteristic pressure tracing

In two patients only was the site of the stenosis estimated on clinical grounds the

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TABLE I Intensity of aortic systolic murmur recorded in the right side of the heart

Case no	Age (yrs)	Diagnosis	Gradient (mm Hg)	Systolic pressure in left ventr (mm Hg)
7101	40	Valv AS + AI + MS	25	148
7043	47	Valv AS + AI	Not meas	Not meas
7289	14	Valv AS	35	155
7299	49	Valv AS	75	170
7440	7	Valv AS	90	190
7506	34	Valv AS + AI	Not meas	220
6281	21	Valv AS	50	157
7564	56	Valv AS + AI	Not meas	Not meas
1773	55	Valv AS	58	179
7474	24	Valv AS	54	157
1496	41	Valv AS	Not meas	230
7604	58	Valv AS	54	170
1296	28	Valv AS + AI	90	205
7369	22	Bic aort valve	None	126
7545	19	Subvalv fibr AS	140	230
7746	18	Subvalv fibr AS+AI	94	193
6548	10	Subvalv fibr AS+AI	90	200
7573	60	Subaort musc S+VSD	116	282
7603	10	Subaort musc S	100	180
6676	22	Subaort musc S	9	120
7309	20	Subvalv AS	Not meas	135
5222	15	Subvalv + valv AS	21	104

Ventr = ventricle Infl = inflow Outfl = outflow Pulm = pulmonary Valv = valvular AS = fibr = subvalvular fibrous VSD = ventricular septal defect Subaort musc S = subaortic muscular catheter was in contact with the atrial septum

murmur was loudest in the 2nd right intercostal space at external auscultation in one patient (case no 1496), in whom a thrill was felt in the suprasternal notch and who also had an ejection click the aortic valve was calcified as seen on the chest roentgenogram This patient was thought to have valvular stenosis The other patient (case no 7309) had no ejection click the murmur had its maximum at the apex and the external carotid artery tracing was normal , this patient was thought to have subvalvular stenosis of unknown type

The systolic aortic murmur in three of the patients (cases no 7604, 1296 and 7746) was a musical murmur (fig 1)

Results

The intensity of the recorded systolic murmur in the superior caval vein, right atrium, inflow tract and outflow tract of the right ventricle, main and

Intensity of systolic intracardiac murmur in the

Superior caval vein (mm Hg)	Right atrium (mm Hg)	Right ventr infl tract (mm Hg)	Right ventr outfl tract (mm Hg)	Main pulm artery (mm Hg)	Right pulm artery (mm Hg)
0 8	0 3	—	—	0 3	0 2
1 0	0 3	0.2	—	0.2	0 4
0 8	0 5	—	—	0 3	0 3
0 5	0 2	—	—	0 3	0 4
1 2	—	—	—	1 0	Not ent
1 8	1 0	—	—	0.3	0 4
0 8	0 4	—	—	0 4	0 3
2 0	1 0	—	—	0.5	0 4
0 4	0 1	—	—	0.2	0 1
0 9	0 7	0 4	0 2	0 3	Not ent
0 7	(1 4)	0 5	—	—	Not ent
0 4	0 3(0 6)	0 3	—	0 3	—
1 0	(1 5)	0 6	0 8	0 7	Not ent
0 4	(0 4)	0 1	0 1	—	0 4
0 6	0 2	1.3	0 7	0 2	0 2
1 3	(1 5)	0 9	—	0 5	0 5
1 0	0 4(1 4)	1 5	0 8	0 8	0 5
—	0 3	0 8	1 0	0 5	Not ent.
0 2	0 3	0 5	1.5	1 3	Not ent
0 2	0 2	0 1	—	0 3	0 5
—	0 7	0 3	—	0.2	0 5
0 1	0.2	—	—	0 3	0 4

aortic stenosis AI = aortic insufficiency MS = mitral stenosis. Bic aort = bicuspid aortic Subv alv stenosis Not meas = not measured Not ent = not entered ( ) intensity when the tip of the

right pulmonary artery of the 22 patients is shown in table I

In the three patients with external musical murmur the intracardiac murmur in all parts of the right side of the heart including the main and the right pulmonary artery was musical on the loud-speaker and showed quite regular vibrations on the recordings (figs 2—5), differing from the recordings of the non musical murmurs (7)

Discussion

In all patients with valvular stenosis the murmur was loudest in the superior caval vein while it was fainter in the right atrium (with the catheter tip lying freely in the cavity) Only in one patient with subvalvular stenosis (case no 7746) was the murmur likewise loudest in the superior caval vein this patient had a musical murmur, and the stenosis was fibrous

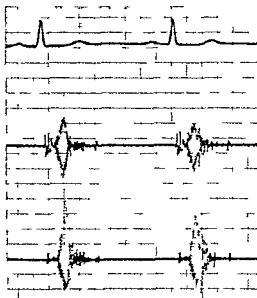


Fig 1 External phonocardiogram from the apex showing musical systolic murmur (case no 7604) (Mingograph 31 B Elema Schonander recording in the *100 hz* and *400 hz* range

In the two remaining patients with fibrous subvalvular stenosis the murmur was loudest in the inflow tract of the right ventricle, but was also rather loud in the superior caval vein Only five patients with valvular stenosis had any murmur in the right ventricle, among those few were the patients with a musical murmur (cases no 7604 and 1296)

In the two patients with severe subaortic muscular stenosis the murmur was most intense in the outflow tract of the right ventricle In the patients with slight subvalvular stenosis the murmur had its maximum at other sites, but common to all three patients in this group was a lack of murmur or only *a faint murmur in the superior* caval vein

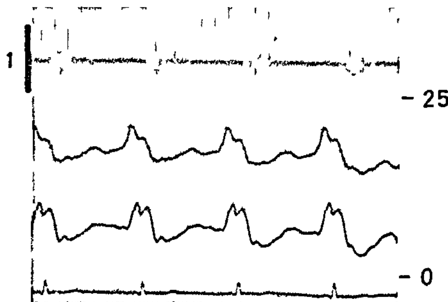


Fig 2 Intracardiac phonocardiogram (upper tracing showing musical murmur in the right atrium, the tip of the catheter is in contact with the atrial septum (case no 7604) In the upper left margin a calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line Pressure is recorded both with the micromanometer at the tip of the catheter (middle tracing) synchronously with the sound and through the side hole (lower tracing) *1.5 cm from the tip the latter tracing being calibrated at 0 and 25 mm Hg*

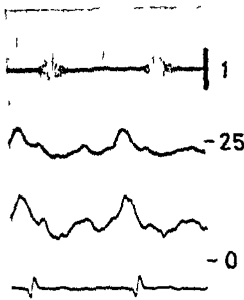


Fig 3 Intracardiac phonocardiogram from the superior caval vein showing musical systolic murmur (case no 1296)

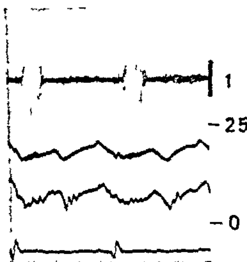


Fig 4 Intracardiac phonocardiogram from the right atrium showing musical systolic murmur and the transmitted diastolic murmur of aortic insufficiency (case no 1296

The patient with bicuspid aortic valve without stenosis resembled the patients with valvular stenosis in respect of the distribution of the murmur

On the basis of these findings it should be possible to use the intensity of the murmur during a right heart catheterization not only as a diagnostic sign (7) of the presence of aortic heart disease, but also in differentiating between the various forms of aortic stenosis In this connection it should be pointed out that the transmission of musical aortic murmurs may differ from the usual pattern

A schematic presentation of the relative intensity of the aortic systolic murmur found in this study is shown in table II It corresponds closely with the findings in a similar study of 26 patients by Reploh and associates (3)

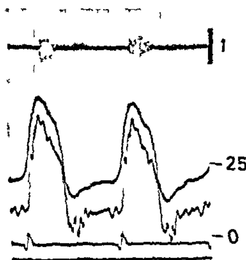


Fig 5 Musical systolic murmur recorded in the inflow tract of the right ventricle case no 1296 The faint diastolic murmur is also seen

TABLE II Relative intensity of the aortic systolic murmur

	Relative intensity of the aortic systolic intracardiac murmur recorded in the					
	Superior caval vein	Right atrium	Right ventr infl tract	Right ventr outfl tract	Main pulm artery	Right pulm artery
Valv AS	[+++]	++	−	−	+	+
Valv AS music m	[+++]	(+)	+	+	++	−
Bic aort valve	[+++]	−	(+)	(+)	−	[+++]
Subvalv fibr AS	++	(+)	[+++]	+	+	+
Subaort musc S	(+)	++	++	[+++]	[+++]	++

Music m = musical murmur The maximal intensity is each group is encircled

Summary

Right heart intracardiac phonocardiography was performed in 22 patients with aortic systolic murmurs

In all patients the murmur could be registered in the right side of the heart The intensity of the murmur at the different sites seemed to be useful in the differentiation between valvular stenosis, subaortic muscular stenosis and fibrous subvalvular stenosis In patients with musical murmurs the transmission to the right side of the heart was slightly different from that in the patients with non musical murmurs

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From the Department of Medicine II (Head E Wassén, M D), University of Göteborg
Göteborg Sweden

Plasma Lipids in Women

Variation in Cholesterol, Phospholipids and Triglycerides at Different Ages in a Random Population Sample

By

L HALLBERG, A M HOGDAHL, A SVANBORG and O VIKROT

Several previous studies both in males and in females have shown an increase in the concentration of different plasma lipids from early childhood until about 50—60 years of age It is not known if this increase with age is directly related to more or less continuous aging processes of the tissues or to changes occurring step by step Such changes may for instance be related to variations in hormonal activities dietary habits, physical activity etc

Most previous studies have been limited to studies in males probably because of the higher incidence of coronary heart disease especially in young men, and because of an assumed relationship between plasma lipid levels and vascular changes The lower incidence of coronary heart disease in young women and the increase in incidence after the menopause makes it especially important to study in detail various changes of the plasma lipid levels with age in women

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The present paper is the first report on a series of studies of plasma lipids in a random population sample of women aged 15 to 75 years To facilitate studies on changes with age, certain age strata instead of age groups have been used

Reports to be published later will include closer analyses of the relationships between plasma lipid levels and e g dietary habits and physical activity As hormonal factors presumably influence plasma lipid levels, special attention will be paid to the plasma lipid changes at the menopause in a forthcoming paper

Material

The present study was made in 360 women selected at random by stratified sampling from the census register of Goteborg The subjects were selected from the following eight age strata 15 23 30 30 40 45 50 60 and 75 years For details of the sampling technique used see Hallberg et al (9)

The number of subjects selected at different age strata the absence of response and the

TABLE I Original population sample Number of subjects at different age strata of the original material and the reasons for absence of response The present study was made in a random sample from this population sample

Age	15	23	30	40	45	50	60	75	Total
Subjects selected	125	125	125	125	123	125	125	125	998
Dead prior to examination	—	—		—	—	1	2	6	9
Moved from the city	3	11	5	9	2	1	1	1	33
Not available at address given	1	1	2	2	—	—	3	3	12
Remaining	121	113	118	114	121	123	119	115	944
Inability to participate because of invalidating disease home care	—	—	—	—	—	—	6	12	18
Hospitalization	1		—	—	3	1	2	11	18
Undergone recent health control unwilling to participate	2	1	—		1	1	1	10	16
Lack of time	4	2	4	4	6	6	6	1	33
Severe social and/or mental insufficiency	—	2	1	2	—	—	1	1	7
Refusal without motive				1	2	1	4	9	17
Subjects examined	114	108	113	107	109	114	99	71	835
Examined as a percentage of remaining	94.2	95.6	95.8	93.9	90.1	92.7	83.2	61.8	88.5
Subjects examined in the present study	45	39	41	33	40	83	40	39	360

reasons for not participating in the study are given in table I The absence of response is small except in the 75-year group

From the 835 subjects examined in the original population study a further random selection of subjects was made for the present study This selection was made after the exclusion of pregnant subjects and subjects who had had a delivery within the preceeding 4 weeks as pronounced changes of plasma lipids occur during pregnancy 18 19 More subjects were choosen from the 50 year group to make possible a closer study on the effects of the menopause The population study was made between March and June 1963

Methods

The subjects were instructed not to eat after 8 p m. and not to drink after midnight on the day before the study Blood was sampled the next morning between 8 and 9 a m

Fifty ml of blood was withdrawn in a plastic tube and was left at room temperature for 4—6 hours after which it was centrifuged Serum was distributed in several small plastic tubes which were immediately frozen and stored at — 20 C for about two years until the analyses were performed

For the lipid analyses 1 ml of serum was added drop by drop to 5 ml of methanol after which 10 ml of chloroform was added Non lipid contaminants were removed from the chloroform phase by the addition of 15 ml of saline and the tubes were left over night This preparation of the lipid extract and the analysis of triglycerides were performed essentially according to Carlson (5) Cholesterol was determined according to Zlatkis et al 20 and phospholipids according to Svanborg and Svennerholm 1961 (17)

TABLE II Control of present cholesterol method. Comparison of 3 commercial cholesterol standards and 4 reference sera using the present cholesterol method. Each value is the mean of 6 determinations.

Cholesterol standards	Reference sera				Value of reference minus value observed M+SE
	Seronorm (mg/100 ml)	Plasma I (mg/100 ml)	Plasma II (mg/100 ml)	Serachol (mg/100 ml)	(mg/100 ml)
NBC[1]	91.5	170.0	247.8	346.4	1.7±5.5
Fisher[2]	92.1	171.0	250.1	352.8	−0.9±6.7
Merck[3]	96.5	177.9	262.1	370.0	−11.0±9.2
Value of reference	104	170	255	333	

[1] Nutritional Biochemicals Corp. Cleveland, Ohio, USA.
[2] Fisher Scientific, Pittsburg, Pa., USA.
[3] E. Merck AG, Darmstadt, Germany.

Comment on the method

Previous investigations have shown that by direct analyses of plasma lipid extracts with the ferric chloride method the cholesterol value will be about 5 per cent too high because of the presence of other compounds than cholesterol. These interfering compounds can be removed by chromatography of the lipid extract on silicic acid (17). In the present population study this time consuming procedure was omitted and the cholesterol values can therefore be expected to be about 5 per cent too high. This was confirmed in a series of 10 seras analyzed both with the method which included silicic acid chromatography (17) and the present method. Further systematic errors are related to the use of different cholesterol standards. The present values are referred to a standard ("cholesterol standard for chemical work") prepared by Nutritional Biochemicals Corp., Cleveland, Ohio. To facilitate a comparison between the results of the present series and those of other series two additional cholesterol standards were included in a comparative study on 4 reference sera: two commercial sera (Seronorm, Nyegaard & Co., Norway, with a concentration of 104 mg cholesterol/100 ml and Serachol, Warner and Chilcott, USA, with a concentration of 333 mg/100 ml) and two pooled patient sera used as standards at the central laboratory of this hospital. The two latter sera had been tested against the above mentioned commercial sera. Their cholesterol concentrations were 170 and 255 mg/100 ml of serum, i.e. concentrations between those of the commercial seras. The mean values of 6 analyses are given in table II which shows that the reliability of the present method was good within the range of the cholesterol concentrations studied.

Results

Mean values and their standard errors at different ages are given in table III. The cholesterol and phospholipid levels increased up to 50 years of age. The cholesterol in the 75 year group was significantly lower than in the 50 and

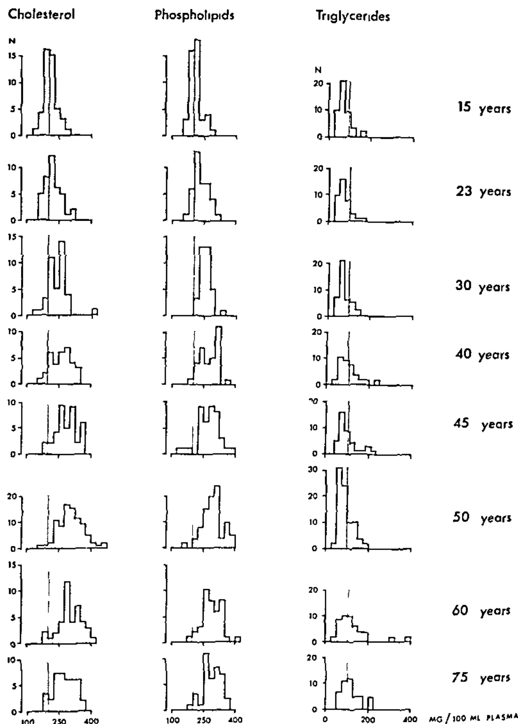


Fig 1 Distribution of cholesterol phospholipids and triglycerides in plasma in women at different ages The dotted lines at 200 mg/100 ml plasma for the cholesterol and phospholipid values and at 100 mg/100 ml plasma for the triglyceride values are graphed to facilitate comparisons between the age groups

TABLE III Plasma lipid levels in women at different ages

Age	No of subjects	Cholesterol (mg/100 ml plasma) Mean value	Cholesterol Standard error of mean	Phospholipids (mg/100 ml plasma) Mean value	Phospholipids Standard error of mean	Triglycerides (mg/100 ml plasma) Mean value	Triglycerides Standard error of mean
15	45	205 3	4 0	210 3	3 8	69 2	4 0
23	39	219 0	5 8	231 0	5 4	71 0	4 2
30	41	236 4	6 8	248 3	5 0	70 8	4 0
40	33	260 2	9 1	275 8	7 1	99 2	6 9
45	40	282 6	8 8	276 3	7 5	89 3	6 9
50	83	309 7	6 3	296 2	5 0	95 1	5 4
60	40	304 1	8 4	291 6	7 2	120 3	10 5
75	39	279 5	8 1	287 7	6 8	118 9	7 3
	360						

60 year groups, but the phospholipids did not decrease significantly

The triglyceride level did not increase continuously with age Thus it was the same in the 15 23 and 30 year groups it was higher and about the same in the 40, 45 and 50 year groups and was still higher and the same in the 60 and 75 year groups, thus showing a stepped increase with age

The cholesterol/phospholipid quotient was less than 1 in patients between 15 and 40 years but more than 1 at the older ages except for the 75 year group

The distribution of the plasma lipid values at different ages is illustrated in fig 1 which shows that single individuals with extreme, high values were not observed in the two lower age groups The ranges of cholesterol and phospholipids increased with increasing age up to 50 years The change of the triglyceride range with age was similar but less pronounced

Discussion

Previous studies reviewed in tables IV—VI, on plasma lipids in females indicate that the concentration of cholesterol and phospholipids increases with age up to 50—60 years and that there may be an increase with age also of the triglyceride level in plasma However only a few studies on plasma triglycerides in females are available As shown in tables IV—VI there were wide ranges of the values within different age groups and usually only small differences between adjacent age groups Part of the wide variations observed in the age groups of earlier studies might be due to a variation with age within the age groups Therefore, in an attempt to diminish the variation within the groups certain age strata instead of age ranges were used in the present study

The age strata studied are probably representative for the population as a

TABLE IV. Review of previous studies on plasma cholesterol in women

Reference	Year	Cholesterol (mg/100 ml plasma) No of subjects	Age	Range	Mean value	Standard deviation	Standard error of mean
Gardner & Gainsbourgh (8)	1927	21	17–25	78–218	153		
Peters & Man (16)	1943	8	10–14		187		
		65	20–50		199		
Auken (3)	1948	50	19–41	143–258	198	31.4	
Kornerup (14)	1950	22	20–39	154–318	224	50	10.6
		15	63–96	163–294	235	35	9.1
Keys et al (13)	1950	564	17–30		177	31	1.3
Josephson & Dahlberg (11)	1952	141	20–50		227	50	4.2
			20–30		235	31	6.9
		54	70		204	35	4.7
Katz et al (12)	1953	12	M 36	166–239	200		
Adlersberg et al (2)	1956	53	13–17		183		4.8
		24	18–22		193		8.8
		40	23–27		202		6.3
		50	28–32		200		4.6
		72	33–37		207		4.5
		64	38–42		225		4.9
		56	43–47		239		6.9
		34	48–52		250		9.1
		28	53–57		286		8.4
		20	58–62		264		14.8
		14	63–67		260		
		3	68–72		242		
Lindholm (15)	1956	21	20–24		164		
		20	30–39		175		
		22	40–49		185		
		18	50–59		193		
		12	60				
		93	20–91	131–241	182		
Svanborg & Svennerholm (17)	1961	28	16–35		185		7.1
Cramér (6)	1962	18	20–40		200		8
		11	55–65		261		9
Feldman et al (7)	1963	13	17–25		206		10.4
		15	26–35		228		9.4
		19	36–45		274		9.1
		19	46–55		280		10.6
		14	56–65		298		16.9
		13	66–75		273		13.5
		6	76–90		281		14.5

Table IV Cont

Reference	Year	Cholesterol (mg/100 ml plasma) No of subjects	Age	Range	Mean value	Standard deviation	Standard error of mean
Johnson et al (10)	1965	284	15−19		173	31	
		243	20−24		189	40	
		311	25−29		194	41	
		350	30−34		197	39	
		328	35−39		207	38	
		253	40 44		219	39	
		208	45−49		224	43	
		163	50−54		238	38	
		150	55 59		249	48	
		90	60 64		255	50	
		95	65−69		258	57	
		69	70−74		253	48	
		41	75−79		231	49	
		39	80		245	49	
		2 624					

whole except for the 75 year group in which the non response was high It is thus impossible to decide if the lower cholesterol and phospholipid levels observed in this latter group are an effect of high age or reflect a selection of healthy individuals The absence of extremely high values in this group may be explained in a similar way, but may for instance also be due to a shorter life span of subjects with extremely high plasma lipid levels

It is tempting to compare the present results with those previously reported Most previous materials except for the Tecumseh study, were not selected at random and comprised mainly healthy individuals For the chemical analysis, different methods have been used in different studies As far as the cholesterol analysis is concerned the different methods and especially the different cholesterol standards used, will make a direct comparison difficult The methods used to determine total phospholipids however will probably give fairly consistent results The 3 studies on plasma triglycerides in women shown in table VI, are all based on a glycerol determination method A comparison between the present population study and the population study in Tecumseh (10) is of special interest In the Tecumseh study cholesterol determinations were made in 2 624 women between 15 and more than 80 years The plasma cho-

TABLE V. Review of previous studies on plasma phospholipids in women

Reference	Year	Phospholipids (mg/100 ml plasma) No. of subjects	Age	Range	Mean value	Standard deviation	Standard error of mean
Auken (3)	1948	7	19−35	139−183	163		
Kornerup (14)	1950	19	20−39	55−268	186	51	11.8
		12	63−96	155−265	208	33	9.8
Adlersberg et al. (2)	1956	46	13−17		236		6.5
		21	18−22		244		9.4
		38	23−27		249		7.3
		45	28−32		241		4.6
		67	33−37		255		4.2
		61	38−42		270		4.5
		50	43−47		275		6.2
		31	48−52		291		7.8
		26	53−57		314		9.9
		18	58−62		298		11.8
		14	63−67		317		
		3	68−72		279		
Lindholm (15)	1956	21	20−24		215		
		20	30−39		240		
		22	40−49		251		
		18	50−59		273		
			60				
		93	20−91	161−403	252	29	
Svanborg & Svennerholm (17)	1961	29			232		8.2
Feldman et al. (7)	1963	13	17−35		246		12.2
		21	35		285		10.8

lesterol level showed a similar increase with age as in the present study, but the absolute level was consistently about 50 mg/100 ml higher in the present study. Therefore, a comparison was made between the cholesterol methods used in the two studies. The mean cholesterol value of ten sera analyzed with the present method was 221 mg/100 ml plasma. Analyzed with the method of Abell et al. (1), which was used in the Tecumseh study, it was 212 mg/100 ml plasma. This difference was statistically significant ($p < 0.01$). The differences between the two studies can thus only partly be explained by the different analytical methods used. However, it is still impossible to determine whether the differences in cholesterol level between the two population materials are real or due to other methodological differences, e.g. the use of different cho-

TABLE VI Review of previous studies on plasma triglycerides in women

Reference	Year	Triglycerides (mg/100 ml plasma)					
		No of subjects	Age	Range	Mean value	Standard deviation	Standard error of mean
Svanborg & Svennerholm (17)	1961	3			88		
Cramer (6)	1962	8	20—40	57—79	68		
		11	55—65	74—93	84		
Feldman et al (7)	1963	13	17—25		61		6 6
		15	26—35		54		3 8
		19	36—45		93		12 8
		19	46—55		95		8 7
		14	56—65		86		7 6
		13	66—75		103		10 8
		6	76—90		120		27 4

lesterol standards (not given for the Tecumseh study)

The phospholipid level in women has been studied only in materials which were not selected at random and which included mainly healthy individuals This may explain the slightly higher phospholipid levels in the present population material

The plasma triglyceride levels are about the same in the present study as in the few previous studies (see table VI) Contrary to what has been observed in healthy men, who have a more continuous increase in the triglyceride level with age up to 46—55 years (4), the plasma triglycerides increased more stepwise in the present material

A closer analysis of the interrelationships between different plasma lipid fractions has not yet been made in the present study

A further analysis of the material with respect to the influence of hormonal and hereditary factors, physical activity, and dietary habits will be accounted for in forthcoming reports Such factors may influence both the absolute plasma lipid levels and the interrelationship between different fractions

The present investigation was made as a basis for prospective clinical studies of this material, which comprises both healthy individuals and subjects with various latent or manifest diseases To evalute the clinical significance of different plasma lipid levels in such studies it is necessary to include all subjects as it is impossible to separate at any given time healthy subjects with latent diseases or evaluate the importance of various symptoms and findings at the time of the first study

Summary

Cholesterol, phospholipids and triglycerides in plasma were determined in

360 non pregnant women selected at random from the census register of Goteborg Certain age strata were studied 15, 23, 30, 40, 45, 50, 60, and 75 years

The cholesterol and phospholipid levels showed a continuous increase with age up to 50—60 years The cholesterol/phospholipid quotient was less than 1 between 15 and 40 years and in the 75 year group, and greater than 1 in the 45, 50 and 60 year groups

The triglyceride level showed a more stepped increase with age The concentration was the same in 15 23 and 30 year old women, it was higher and about the same in the 40 45 and 50-year groups and still higher and the same in the 60 and 75 year groups

The influence of various factors except for age, on the plasma lipid level will be reported in forthcoming papers

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From Department IX (Head K Aas M D) Ullevål University Hospital Oslo Norway

Rapid Elimination of Evans Blue in Amyloidosis

By

U Abildgaard

Rapid plasma elimination of Evans blue in patients with amyloidosis was first reported by Unger et al (13) In 1960, Jarnum (8) suggested that a more rapid elimination of Evans blue than of I^{131} albumin might be diagnostic of amyloidosis

In two recent studies the elimination of these two substances was compared Whereas Cathart and Cohen (4) found rapid elimination of Evans blue in only one of six patients, Larsen and Jarnum (9) found rapid elimination in twelve of eighteen patients

Because equipment for the simultaneous determination of I^{131} elimination was not available when this study was initiated Evans blue elimination alone was examined To increase the diagnostic value a multiple blood sampling procedure was followed As a slow elimination of Evans blue in normal individuals seems well established (6 7, 9,) the present control group comprised patients in which increased elimination might be suspected because of circulatory failure (6), or various protein disturbances other than amyloidosis

Submitted for publication May 23 1966

Ten of thirteen patients with amyloidosis eliminated Evans blue more rapidly than these controls

Material and methods

Patient material

1 Patients with amyloidosis (thirteen cases) A histological diagnosis was obtained in all cases (table I) Biopsy was positive in nine cases and post mortem examination revealed amyloidosis of the liver spleen and kidneys in ten cases In six patients amyloidosis coexisted with myelomatosis in the remaining cases it was secondary to tuberculosis rheumatoid arthritis and spondylarthritis ankylopoietica

2 Patients without amyloidosis (eighteen cases) In nine of these controls no evidence of amyloidosis was found at post mortem examination (table II) After a thorough clinical examination and evaluation it was concluded that amyloidosis could probably be excluded in the remaining nine

Methods

The patients were fasted and kept recumbent for eight hours prior to the test Blood samples (6—8 ml) were drawn into heparinized tubes the degree of venous congestion

TABLE I Patients with amyloidosis

Age	Sex	Diagnosis	Histological diagnosis of amyloidosis		Evans blue test (%)[1]
			Biopsy	Autopsy	
51	♀	Chronic pulmonary tuberculosis Spondylarthritis ankylopoietica	Rectal pos	Pos	49
60	♀	Chronic pulmonary tuberculosis		Pos	22
47	♂	Chronic pulmonary tuberculosis	Liver pos		2
42	♂	Chronic pulmonary tuberculosis	Rectal pos	Pos	4
47	♂	Rheumatoid polyarthritis Spondylarthritis ankylopoietica		Pos	35
43	♀	Spondylarthritis ankylopoietica	Liver gingival neg Renal pos	Pos	29
62	♀	Rheumatoid arthritis		Pos	18
66	♀	Rheumatoid arthritis Diabetes mellitus Myelomatosis	Rectal pos	Pos	2
76	♀	Myelomatosis		Pos	36
55	♂	Myelomatosis	Rectal pos Renal neg	Pos	18
59	♀	Myelomatosis	Inguinal tumour pos		20
49	♂	Myelomatosis	Lumbar vertebra pos		32
52	♀	Myelomatosis	Liver pos	Pos	27

[1] Evans blue test The decrease in plasma concentration between 5 and 30 minutes after injection
All these thirteen patients had proteinuria

being kept to a minimum A control blood sample was drawn before injection of Evans blue (about 15 mg) into an arm vein Five minutes later the first sample was drawn from a vein on the opposite arm The needle was kept open by injections of saline every minute and five more samples were drawn at five minute intervals To avoid dilution with saline the first ml of blood was discarded Usually all samples could be drawn through the same needle but occasional samples were lost because of clotting

The blood samples were centrifuged at 2 000 g for 30 min If the plasma was not clear it was centrifuged at 28 000 g for 60 min After this second centrifugation even lipemic plasmas (table I patients no 6 and 12) were clear in accordance with the findings of Burstein (3) The concentration of Evans blue was determined with a Beckman B spectrophotometer at 620 mμ using the patient s control plasma as blank In patients with rapid elimination of Evans blue the dye could not be demonstrated in the urine

Evans blue A 0 5 per cent aqueous solution from Warner Chilcott Division Morris Plains New Jersey, U S A

Heparin A 5 per cent (w/v) solution containing 100 i u per mg heparin from AL, Oslo Norway was used for heparinizing the syringes

Evans blue test The decrease in plasma concentration between 5 and 30 min after injection

TABLE II Patients without amyloidosis

Age	Sex	Diagnosis	Proteinuria	Autopsy performed	Evans blue test (%)[1]
61	♂	Bronchiectasiae Cor pulmonale Chronic pyelonephritis	+	+	13
57	♂	Emphysema Cor pulmonale	+	–	5
74	♀	Cardiosclerosis	–	–	12
81	♀	Cardiosclerosis Rheumatoid arthritis	–	–	3
58	♂	Chronic pyelonephritis Hypertension Congestive hepato-splenomegalia	+	+	5
46	♀	Chronic glomerulonephritis	+	–	7
70	♂	Emphysema Malabsorption Atrophic ileitis (biopsy)	–	–	4
62	♂	Sideropenic anemia Mesenteric lipogranulomata (biopsy)	–	–	11
58	♂	Chronic lymphatic leukemia Coronary heart disease	–	–	13
61	♀	Panarteritis nodosa	+	–	12
41	♂	Cirrhosis of the liver Chronic alcoholism	–	–	11
52	♀	Myelomatosis	+	+	5
70	♂	Myelomatosis	+	+	10
59	♂	Macroglobulinemia Waldenstrom	+	+	7
56	♀	Macroglobulinemia Waldenstrom	+	+	6
67	♀	Adenocarcinoma of the stomach with metastases	–	+	5
58	♀	Carcinoma of the pancreas with metastases	–	+	9
56	♂	Cholangiohepatoma with metastases	–	+	15

[1] Evans blue test The decrease in plasma concentration between 5 and 30 minutes after injection

TABLE III Distribution of the decreases in plasma concentration of Evans blue between 5 and 30 minutes after injection

	Amyloidosis	Controls
Decrease in concentration per cent	2 2 4	3 4 5 5 5 5
		6 7 7 9
		10 11 11 12 12 13 13
	18 18	15
	20 22 27 29	
	32 33 35 36	
	49	
Mean value	22.9	9.4
Standard deviation	13.9	3.8

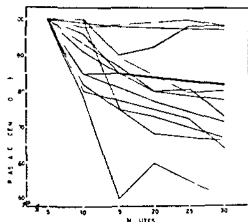


Fig. 1. Plasma concentration of Evans blue in patients with amyloidosis. The concentration in the sample drawn 5 minutes after injection was given a value of 100 per cent.

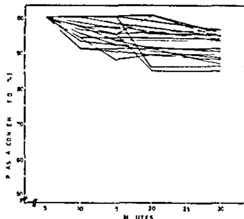


Fig. 2. Plasma concentration of Evans blue in controls. The concentration in the sample drawn 5 minutes after injection was given a value of 100 per cent.

Results

The individual test results are listed in table I (amyloidosis) and table II (controls). In the eighteen controls (table III), the decrease in Evans blue concentration ranged from 3 to 15 per cent with a mean value of 9.4 per cent and a standard deviation of 3.8 per cent. Assuming a normal distribution of the results, the probability is about 0.05 of obtaining a decrease larger than 16 per cent and about 0.01 of obtaining a decrease larger than 19 per cent.

In contrast, of the thirteen patients with amyloidosis only three patients had decreases less than 16 per cent and five patients less than 19 per cent.

Figs. 1 and 2 show that in nearly all patients, the greater part of the decrease occurred during the initial 15 minutes. In the ten patients with rapid elimination, the curves differed most from those of the controls during the initial 15 minutes.

Discussion

Although only in amyloidosis has rapid elimination of Evans blue been reported, elimination may be moderately increased in some pathological states. In conditions with frank leakage of plasma from the vascular bed (exudative peritonitis and extensive burns), disappearance rates averaged 15 per cent during the first hour but never exceeded 25 per cent (7). In other groups of patients (11, 12), and in normal individuals (6, 7), disappearance rates were 3—11 per cent.

A slightly increased elimination of Evans blue in chronic infectious diseases was observed by Larsen and Jarnum (9). Although rapid elimination has not been reported in malignant disease, this might be suspected since Duran—Reynals (5) observed accumulation of Evans blue in the stroma of malignant mice tumours.

The steeper decrease in concentration observed during the first few minutes after injection has been attributed to

mixing within the capillary bed, and this part of the curves should not be used for calculation of the disappearance rates (6) Some investigations seem to indicate that even the initial, steeper fall in concentration is partly caused by extravascular passage of the dye (1, 10) Also in the control patients in this study, the concentration decreased most during the first 15 minutes, but by not more than 15 per cent during the test period A decrease exceeding 19 per cent should therefore strongly indicate amyloidosis

If disappearance rates were calculated from the second, straight part of the curves (4), only a small difference between the groups existed This confirms that examination during the early period after injection is important (8) To reduce errors due to incomplete mixing the first samples were drawn after five minutes, instead of four minutes as employed by Jarnum (8) For the same reason as well as to reduce the influence of any eventual error of determination, multiple blood sampling was used

In eight of the thirteen patients with amyloidosis concentration decreased more than 19 per cent This is a lower proportion of positive results than obtained by Blum and Sohar (2) with rectal biopsy, but similar to that of Larsen and Jarnum (9) who compared Evans blue and I^{131} albumin elimination The latter method will tend to reduce errors from incomplete mixing, but may perhaps increase methodological variation The present data indicate that determination of Evans blue elimination alone may be a useful test for amyloidosis

Summary

The concentration of Evans blue in plasma has been determined repeatedly by a first sample drawn five minutes and a sixth thirty minutes after intravenous injection In three patients with amyloidosis, and in the eighteen controls, the concentration decreased by 15 per cent or less during this period In the remaining ten patients with amyloidosis, an increased elimination was found, ranging from 18 to 49 per cent In these ten patients the concentration curves differed most from those of the controls during the initial 15 minutes

It is therefore concluded that determination of Evans blue elimination may be of diagnostic aid in this condition A decrease in concentration during the test period exceeding 19 per cent can probably be regarded as diagnostic of amyloidosis

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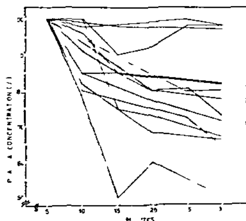


Fig 1 Plasma concentration of *Evans blue* in patients with amyloidosis. The concentration in the sample drawn 5 minutes after injection was given a value of 100 per cent

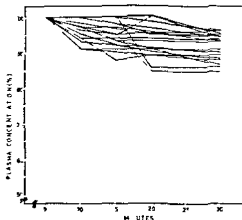


Fig 2 Plasma concentration of Evans *blue* in controls The concentration in the sample drawn 5 minutes after injection was given a value of 100 per cent.

Results

The individual test results are listed in table I (amyloidosis) and table II controls) In the eighteen control table III), the decrease in Evans blue con centration ranged from 3 to 15 per cent, with a mean value of 9 4 per cent and a standard deviation of 3 8 per cent Assuming a normal distribution of the results, the probability is about 0 05 of obtaining a decrease larger than 16 per cent and about 0 01 of obtaining a decrease larger than 19 per cent

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Discussion

Although only in amyloidosis has rapid elimination of Evans blue been reported, elimination may be moderately increased in some pathological states In conditions with frank leakage of plasma from the vascular bed (exudative peritonitis and extensive burns), disappearance rates averaged 15 per cent during the first hour but never exceeded 25 per cent (7) In other groups of patients (11 12) and in normal individuals (6 7), disappearance rates were 3—11 per cent.

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The steeper decrease in concentration observed during the first few minutes after injection has been attributed to

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From Medical Department B (Head A Tybjærg Hansen) Rigshospitalet (University Hospital), Copenhagen Denmark

The Anti-arrhythmic Effect of Propranolol

By

ALF WENNEVOLD and ERIK SANDOE

In recent years propranolol (Inderal) — a beta receptor antagonist (1) — has been used in the treatment of cardiac arrhythmias (3 5, 7, 10, 11, 15), angina pectoris (4, 6, 8, 13), and hypertension (9)

We started using propranolol in the treatment and prophylaxis of arrhythmias in November 1964 Pronethalol (Alderlin), which was the first beta blocking agent used clinically, had turned out to be vitiated by serious side effects Thus the new drug propranolol was used mainly in patients in whom other treatment had been ineffective Consequently our patient material is rather selected

In this paper are described our experience with propranolol in the treatment and prophylaxis of arrhythmias and the side effects which we have observed

Material and method

During the period from November 1st 1964 to March 1st 1966 15 patients were treated with propranolol (table I) Two of the patients were children of 8 and 12 years of age The remaining patients were adults their ages ranging from 20 to 64 years

The indication for the treatment and the daily dosage of propranolol are shown in table I Four patients (nos 5 13 14 and 15) were treated with the object of stopping a prolonged attack of arrhythmia In the remaining cases treatment was instituted in order to prevent episodes of arrhythmia In four patients (nos 1—4) — among them the two children — the treatment was continued for one year or more The remaining patients were treated for periods varying between a few hours and 7 months (table I)

None of the patients was in heart failure at the institution of treatment

Results

Table I reviews the patients in whom an effect was obtained The following case reports serve to illustrate the effect of propranolol

Patient no 1 Female born 1956 For a couple of years she had suffered from Adams Stokes attacks because of bouts of ventricular extrasystoles on exertion No organic heart disease was found (16) Treatment with propranolol was instituted and the effect of the drug

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TABLE I Survey of propranolol therapy in 15 patients

No	Sex	Age (yrs)	Indication	Daily dosage (mg)
1	♀	8	Adams Stokes attacks	40
2	♀	12	Adams Stokes attacks	80—30
3	♀	48	Par ventr tach	120—20
4	♂	49	Par ventr tach	40—80
5	♀	25	Suprav tach	40—30
6	♀	57	Par suprav tach	60
7	♀	36	Par suprav tach	40
8	♂	20	Par suprav tach	90—120
9	♀	41	Par atrial fibrillation	40
10	♂	59	Par atrial fibrillation	40
11	♀	64	Atrial fibrillation[1]	80—40
12	♀	53	Par atrial fibrillation	15—30
13	♂	54	Extrasystoles	40—60
14	♂	62	Intermittent ventr tach	20
15	♂	38	Atrial fibrillation	40

Par ventr tach = paroxysmal ventricular tachycardia Suprav = supraventricular

[1] For the maintenance of sinus rhythm after electric conversion of atrial fibrillation

on the electrocardiogram during exercise was followed (17) She had received 10 mg four times daily for 16 months Since institution of treatment there have been no attacks and no side effects have been observed

Patient no 2 Female born 1952 who developed cardiac syncopes on exertion a few months after a severe attack of measles She had persistent bradycardia at rest (40—48/min), but on exertion bouts of multifocal extrasystoles occurred and tachyarrhythmia had also been recorded during an attack In spite of treatment with many different agents the attacks became more frequent over several years and were now provoked also by emotional disturbances

Treatment with propranolol was instituted and the effect on the electrocardiogram during exercise was followed (17) During the next 13 months the dosage was reduced gradually from 80 mg to 30 mg daily because when a constant dose was given, she would become tired and drowsy several times at intervals of months and at the same time the bradycardia became more pronounced and several brief syncopal attacks occurred The drowsiness disappeared completely as soon as the drug had been withdrawn for 24 hours After treatment for three months with 30 mg daily she had three attacks at intervals of one week the third of these attacks lasting for 5—10 min and being accompanied by convulsions The pulse rate at rest was 30/min The treatment was discontinued for some days and as usual she soon became more alert but afterwards three attacks occurred at intervals of one day Treatment has now been resumed with a dosage of propranolol of *10 mg three times* daily except on Sundays

Patient no 3 48 year old female with severe attacks of ventricular tachycardia for about two years The attacks became more frequent and increasingly difficult to treat and

Duration of treatment	Effect	Cause of withdrawal	Side effects
16 months	+		
16 months	(+)		Tiredness thrombocytopenia
14 months	+		Sinoauricular block dizziness tiredness weight gain
12 months	(+)		Dizziness tiredness thirst sensation of cold
42 days	+	Side effect	Nausea asystole tiredness
7 months	+	Side-effect	Tiredness
15 days	0	No effect	
19 days	0	No effect	
4 months	+		
14 days	0	Aggravation	Nausea more frequent and prolonged episodes
46 days	(+)	Transitory effect	Heart failure
4½ months	(+)	Transitory effect	Atrial tachycardia with 2 1 block
9 days	+	Death	
1 dose	+	Death	
2 doses	0	Death	

many different drugs have been given for prophylactic purposes without any effect Even on minor exertion multifocal extrasystoles occurred (fig 1) which on heavy exertion changed into ventricular tachycardia

During treatment with propranolol the tendency to extrasystoles on exertion diminished (fig 1), and since the institution of treatment no episodes of tachycardia have been observed However the heart rate has become slower with a tendency to periods of sino-auricular block with dizziness (fig 1) During the last 14 months this has necessitated a continuous reduction in dosage from 30 mg four times daily to the present dosage of 10 mg twice daily An attempt to reduce the dosage to 10 mg daily has been made but on this dosage the number of exertional extrasystoles was very troublesome On the present dosage there are neither too many extrasystoles nor any severe episodes of dizziness

Apart from a brief period of tiredness no untoward effects have been observed However during the last year the patient has gained 6—7 kg in weight

Patient no 4 49 year old male with attacks of ventricular tachycardia for four years provoked by emotional disturbances For 3—4 months the attacks increased in frequency they could no longer be stopped by the usual antiarrhythmic drugs but as for later attacks they had to be treated with el conversion On a dosage of 15 mg propranolol four times daily an isolated attack occurred after 2 1/2 months then another attack occurred one month later when the patient tried to reduce the dosage to 15 mg three times daily because he had a feeling of lightheadedness Now he continued on 15 mg four times daily without any complaints and without attacks for 4 1/2 months In connexion with an attack which occurred after this period a subendocardial *myocar*

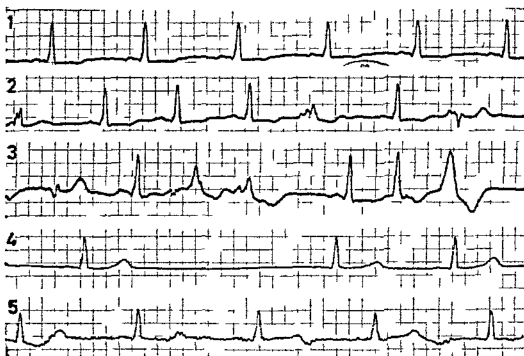


Fig 1 Electrocardiogram taken by means of telemetry in an untreated 48 year-old female (patient no 3) (1) at rest (2 while the patient washed her face and (3) while walking fast Multifocal extrasystoles are observed on exertion The following day 2 1/2 hours after ingestion of 30 mg of propranolol the electrocardiogram shows at rest (4) a slower heart rate and sinoauricular block while walking fast (5 no extrasystoles are observed Paper speed 50 mm sec

dial infarction was diagnosed During the following month there were another two attacks in spite of unchanged dosage and consequently the dosage was increased to 20 mg four times daily and — after another attack two weeks later — to 30 mg four times daily On this dosage the patient complained of tiredness dizziness and sensation of cold and dryness Bradycardia has not been observed

Side-effects and complications

During the treatment three patients died suddenly Their case reports are stated below

Patient no 13 54 year-old male with posterior wall infarction 6 years previously Since then he had been troubled by irregular alternations of rapid and slow heart action For one year there was increasing exertional dyspnoea and frequent nocturnal episodes of dyspnoea A few weeks before admission he had an Adams Stokes attack During his stay in hospital there were numerous extrasystoles in particular on exertion and neither digitalis nor quinidine caused any improvement of the condition Then the patient was given propranolol 10 mg four times daily for six days and 15 mg four times daily for three days The extrasystoles became less frequent but he felt increasingly tired Three hours after the third dose of 15 mg propranolol on the ninth day asystole set in and attempts at resuscitation failed Autopsy revealed considerable cardiac enlargement in particular of the left ventricle and an old posterior wall infarction

Patient no 14 62 year-old male with a three week old anterior wall infarction admitted because of regularly occurring bouts of

ventricular extrasystoles (each normal complex being followed by 4—5 extrasystoles cf fig 2) He was cool and perspiring somewhat confused with slight cyanosis of the lips but his systolic blood pressure remained constant around 150—165 mm Hg In the first instance polarizing treatment (12) was given with glucose potassium chloride and insulin for five hours without the slightest effect on the arrhythmia Then 20 mg of propranolol was administered orally As appears from fig 2 the number of extrasystoles decreased gradually until only sinus rhythm remained However, two hours later asystole set in resuscitation procedures were started immediately, but were unsuccessful Autopsy revealed extensive anterior wall and septal infarction

Patient no 15 38-year-old male in whom mitral valvulotomy had been carried out one year previously because of severe mitral stenosis On admission he had a rapid atrial fibrillation which was difficult to treat Digoxin was given in relatively large doses for one month Since the ventricular action was still rapid two doses of 20 mg propranolol were given on the suspicion of digitalis intoxication Eight hours after the last dose was given cardiac arrest occurred and attempts at resuscitation failed Autopsy revealed enlargement of the heart and severe aortic stenosis

Congestive heart failure developed in one patient but disappeared on reduction of the dose Two patients complained of transitory nausea (nos 5 and 10) Increase in weight was observed in one patient (no 3) Finally one patient complained of thirst and weakness, and of sensation of cold and dizziness (no 4)

After treatment for 2—12 weeks four patients (nos 2 3 5 and 6) noticed increasing fatigue and the heart rate became slower necessitating a reduction in dose or discontinuance of treatment for a period Nocturnal bradycardia was provoked by the treatment in one patient (no 5) and persisted till 13 days after the treatment was discontinued as will appear from the following case report

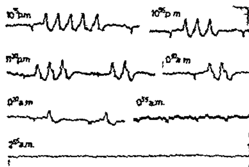


Fig 2 Electrocardiogram (standard lead III) in a 62 year-old male with myocardial infarction (patient no 14) It will be seen that one sinus beat is followed by five ventricular extrasystoles At 10 15 p m 20 mg propranolol were given orally Fourty minutes later the ventricular extrasystoles disappeared one by one so that 2 hours and 40 minutes after the ingestion only sinus rhythm was seen Two hours later asystole set in Paper speed 25 mm/sec

Patient no 5 25 year-old female with persistent intractable sinus tachycardia for about 5 years at a rate of 160—180 per minute the cause being unknown

Treatment with propranolol in doses of 20 mg four times daily had no effect on the heart rate for the first 16 days After that time the heart rate was constantly 48—64 per min throughout the night 100 per min when the patient woke up in the morning 130—140 per min one hour later 160 per min in the late afternoon Then it fell again to 100 per min at about 8 p m and to 60 per min at 10 p m

This diurnal rhythm persisted for approximately one month in spite of reduction in dosage to 10 mg four times daily and 10 mg three times daily The patient was uncomfortable when the pulse rate was low with dizziness and tension behind the eyes and when several brief episodes of asystole occurred propranolol treatment was discontinued

However the peculiar diurnal rhythm persisted unchanged for the next 13 days when the pulse rate suddenly changed to a fairly constant level of about 140 min and afterwards slowly reached its previous level

In one of the patients (no 2) transitory thrombocytopenia was observed (to 40 000/μl) No other disturbances in the blood picture the liver or kidney function were observed in any patient and moreover the two children developed normally

Discussion

In some patients propranolol may depress the irritability of the myocardium effectively and thereby prevent or reduce the number of extrasystoles or other, more serious types of tachyarrhythmias (5, 10, 11) Our findings are in accordance with those of other authors, since an effect was demonstrable in 11 out of 15 patients, although this effect was only transitory in four of the patients

Concurrently with the depression of the irritability, a reduction in the heart rate may occur (5, 10) so that bradycardia accompanied by symptoms may develop, possibly associated with a sinoauricular block (patient no 3) This and the decreasing tolerance to the drug which was observed in a few of our patients may necessitate a maintained reduction in dosage or perhaps intermittent treatment, and calls for a regular follow-up of all patients

It has been shown by animal experiments that beta receptor blockade reduces the contractibility of the myocardium (2), and corresponding to this phenomenon development of heart failure has been observed (9, 14) As stated above, this happened in one of our patients

As for any other potent antiarrhythmic drug, the administration of propranolol involves the risk of development of asystole Of course, this risk is particularly pronounced in patients with a weak myocardium In two of the three patients in our material who died suddenly, an electrocardiogram was obtained and asystole was demonstrated Therefore, propranolol may have been a contributory cause of death, although both these patients were in a very poor condition, and the fatal course could easily be explained by other factors, the dosage of propranolol being low In the third patient (no 15), the cardiac arrest occurred so late after the administration of propranolol that any direct causal relationship must be reckoned hardly probable

In respect of the size of the dose, we used from moderate to medium amounts of the drug, not exceeding 120 mg daily, divided into 3 or 4 doses In the literature amounts of up to 400 mg daily are reported (4, 9)

It is concluded that in some patients propanolol is an effective antiarrhythmic drug, but that it may lead to severe side effects such as bradycardia, sino auricular block, heart failure, and — especially in patients with a weak myocardium — to death because of asystole It may be difficult to find a suitable balance of treatment, and frequent control including electrocardiography is therefore required when this drug is given

Summary

Fifteen patients with various types of arrhythmia were treated with propranolol (Inderal) In 4 patients — among them two children aged 8 and 12 years — the treatment was continued for one year or more

In 11 of the patients the tendency to arrhythmia was reduced or disappeared completely during treatment, in 4 of the cases however, this effect was only transitory

Side effects such as bradycardia sino-auricular block, and — especially in patients with a weak myocardium — asystole were observed One patient developed heart failure In four cases the sensitivity to propranolol increased and a gradual reduction in dosage became necessary

Three patients with a severe heart disease died suddenly, and the deaths could be related in time to the admin istration of propranolol In two of the cases an electrocardiogram was taken at the onset of the heart arrest and asystole was observed

Acknowledgement

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From Medical Department A (Head P A Owren M D), and the Institute for Thrombosis Research (Head H Stormorken M D), University Hospital Rikshospitalet Oslo Norway

Intravascular Coagulation, a Possible Accelerating Effect of Prednisone

By

SVERRE BLIX and CARL DITLEF JACOBSEN

It is well established that the defibrination syndrome may occur in the course of various diseases (16) The syndrome may appear in an acute or chronic form probably triggered by different mechanisms

We have previously studied the possible connection between haemangiomas and intravascular coagulation (2, 3, 5, 6), and the present report adds new evidence to this hypothesis

A patient was admitted to our department with a bleeding tendency, and the laboratory data revealed a defibrination syndrome The diagnosis at autopsy was angiosarcoma of the spleen The intravascular coagulation increased synchronously with two periods of prednisone treatment and this might indicate an influence of corticosteroids on the defibrination process

Case report

A 66-year old woman successfully treated for thyrotoxicosis in 1959 was admitted to the hospital December 1964 due to splenomegaly melaena thrombocytopenia and anemia Splenomegaly was pronounced and the liver was somewhat enlarged There were large ecchymoses on the truncus However, the intestinal bleeding had stopped on admittance

Hb 11 7 g/100 ml R bl c 4 06 mill Leucocytes 12 500 normal diff count Platelets 42 700/μl Bone marrow showed unspecific changes Serum values urea 38 mg/100 ml Creatinine 0 9 mg/100 ml Alkaline phosphatase 6 8 Bodansky units Bromsulphalein retention 5 % (45 min) Bilirubin 0 4 mg/100 ml Cholesterol 260 mg/100 ml SGOT 26 units SGPT 14 units Iron 74 μg/100 ml Total protein 7 2 g/100 ml with normal electrophoretic pattern TT 72 %, Fibrinogen 200 mg/100 ml The urine was normal Bleeding time (Ivy) 16 min

Our first tentative diagnosis was thrombocytopenic purpura and in an attempt to stop the bleeding tendency prednisone therapy (40 mg daily) was started one week after admission However the number of platelets decreased and a fibrinogenopenia became evident Blood values suggested a defibrination syndrome (table I) After ten days the dose of prednisone was reduced and the therapy was discontinued about two weeks later During the period of predni-

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TABLE I Hematological values on admission (4/12), ten days later (14/12) and after the recording of clotting factors had been started Prednisone therapy was given from 8/12

1964	4/12	14/12	18/12
Fibrinogen (mg/100 ml)	200	20	70
Prothrombin (%)			78
Factor V (%)			60
Factor VIII (%)			70
Proactivator (%)			76
Platelets (per μl)	42 700	18 900	11 500

sone reduction the fibrinogen started to increase and after the therapy had been stopped platelets rapidly rose to normal values

About three weeks later prednisone again was given resulting in new sign of intravascular coagulation Treatment was then discontinued after ten days and all signs of defibrination disappeared

Attempts to establish a correct diagnosis of the primary disease failed but when the number of platelets became normal a puncture biopsy of the spleen was performed and the cytology suggested a malignant tumor At the same time an effusion in the left pleura appeared possibly due to metastases However splenectomy was performed and the weight was 1 300 g Microscopically the diagnosis was probably an angiosarcoma and fibrin deposits were seen on the vessel walls (fig 1) A few days after the operation metastases were found in the left humerus and she died three weeks later

A detailed survey of some of the more important hematological values in relation to the prednisone therapy is given in figs 2 and 3

The autopsy findings infiltration of angiomatous tumors in liver and in lymph nodes in liver hilus, in right lung left adrenal cortex left humerus costae and columna There was also a hemorrhagic effusion in the left pleura small amounts of ascites and a probable cortical adenoma beside the metastases in the left adrenal cortex The final diagnosis was angiosarcoma in the spleen with metastases



Fig 1 Histological sections of the spleen A At low magnification the tumor tissue appears cellular with numerous sinus like lumina with atypical endothelial cells H E (Init magn 25 ×) B High magnification reveals that the sinus like spaces are partly filled with blood Note the atypia of the lining cells H E (Init magn 160 ×) C In a section stained with P T A H coarse strands of fibrous material staining like fibrin fill the sinus like spaces in several areas of the tumor (Init magn 64 ×) (Biopsiavd Rikshospitalet)

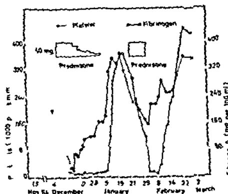


Fig 2 Fluctuations in fibrinogen and platelet values in relation to treatment with prednisone

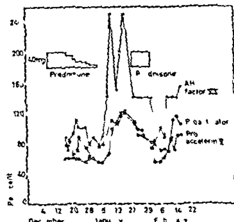


Fig 3 Factor VIII factor V and proactivator fluctuations in relation to treatment with prednisone

Laboratory investigations

1 Methods

Collection of blood Blood samples were collected before breakfast every second or third day during the observation period Nine parts of blood were mixed with one part of a sodium citrate dihydrate solution (3 13 g/100 ml) in ice bath The blood was immediately centrifuged at 4° C for 30 min at 2 500 rpm (1 400 *g*) to obtain platelet poor plasma and the plasma pipetted off and stored in aliquots at —20 C. All clotting and fibrinolytic tests were performed at the same time for each series of assays Platelet rich plasma was obtained by centrifuging the blood at 4° C for 30 min at 600 rpm (80 *g*)

Factor II Modification of the method of Hjort et al (12)

Factor I One stage assay with human brain thromboplastin and a stored human plasma substrate prepared according to Stormorken (19)

Factor VII One stage assay with human brain thromboplastin adsorbed ox plasma to supply added factor V and hereditary factor VII deficiency plasma (11)

Factor VIII A time based assay for activated partial thromboplastin with a cephalin kaolin reagent and with as substrate plasma deficient in hereditary factor VIII (18)

Factor IX A partial thromboplastin time assay with, as substrate plasma deficient in hereditary factor IX and with a cephalin reagent (18)

Factor X Modification of Hougie s method (13) in which cephalin serves as the lipid and the incubation time from addition of the venom is exactly 3 min

Fibrinogen was determined as fibrin after addition of epsilon amino-caproic acid and coagulation with thrombin by the method of Jacobsson (15) modified by Blomback and Blomback (8) and Godal (10)

Fibrinolysis was tested on standard fibrin plates (1)

Inhibitors of fibrinolysis were tested by the method of Blix (7

Plasminogen was determined by the method of Jacobsen 14

Platelets were counted by the method of Nygaard 17)

Platelet sensitivity As prednisone is insoluble the sensitivity to corticosteroids was tested in vitro in three ways after addition of one ml of Actocortin Cortec containing 0 1 mg sodium hydrocortisone phosphate) to one ml of platelet rich

TABLE II The values for prothrombin factor VII factor IX factor X fibrinolysis plasminogen and inhibitors of fibrinolysis during the investigation period

		Prothrombin (%)	Factor VII (%)	Factor IX (%)	Factor X (%)	Fibrinolysis (mm^2)	Plasminogen (%)	Inhibitors (%)
Dec	17	92	105	165	98	0	72	85
	18	78	62	90	98	0	86	85
	19	76	83	100	86	0	86	105
	21	74	110	90	86	0	66	85
	23	80	96	110	82	0	86	85
	24	94	116	125	86	0	92	80
	28	84	100	165	86	0	60	85
	30	76	116	125	98	0	69	80
Jan	2	90	134	145	104	0	88	85
	4	82	100	110	92	0	63	85
	6	76	116	90	112	0	50	80
	9	76	80	100	98	0	50	105
	11	80	90	145	130	0	62	120
	14	100	100	165	130	0	57	95
	16	100	116	165	140	0	69	110
	18	116	110	180	112	0	75	95
	23	108	90	165	112	0	67	120
	25	112	116	165	98	0	84	—
	27	100	100	125	98	0	73	95
Febr	1	112	110	165	98	0	58	80
	3	84	90	110	98	0	49	95
	6	94	80	145	98	0	62	100
	8	88	80	165	98	0	58	105
	10	90	80	165	120	0	60	100
	13	100	90	165	112	0	67	105
	15	100	96	165	104	0	65	85
	17	94	90	165	98	0	—	120

plasma (platelet agglutination release of platelet factor III and clot retraction)

Proactivator was determined by the method of Blix (4)

2 Results

Concerning prothrombin, factor VII factor IX, and factor X, no significant variations were observed There were no systematic changes in plasminogen or inhibitors of fibrinolysis, and no increased fibrinolytic activity at any time (table II)

The results of platelet and fibrinogen investigations are given in fig 2, and fig 3 shows the concentrations of factor VIII, factor V, and proactivator of the fibrinolytic system

No platelet sensitivity to corticosteroids was revealed

Discussion

In the present patient most of the criteria of intravascular coagulation has been found including decreased levels of fibrinogen factor V and factor VIII and a low platelet count The decreased proactivator of the fibrinolytic system is likewise compatible with the defibrination syndrome (4) As is seen in fig 3 there is a remarkable correlation between the proactivator and factor V values Only the prothrombin values were not consistent with intravascular coagulation

In our patient there were two periods of defibrination corresponding to prednisone therapy Between and after these periods high factor VIII and fibrinogen values indicated a hypercoagulable state in the blood (9) This could have been coincidental but could indicate that corticosteroids might induce the defibrination syndrome in patients who are in a hypercoagulable state We have found the platelet count to be a sensitive indicator of the defibrination process It was not possible to reveal platelet sensitivity to corticosteroids by in vitro techniques

As previously emphasized (2 5) anticoagulant treatment seems to be the correct therapy in similar cases but in this patient the intravascular coagulation ceased spontaneously with stoppage of the prednisone treatment.

Summary

More evidence is added to the hypothesis that there is a connection between hemangiomas and the defibrination syndrome In a patient with hemangio-sarcoma in the spleen and a defibrination process, the latter might have been intensified by the administration of prednisone.

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11 [illegible] P [illegible] blood [illegible] plasma Scand J [illegible] Lab [illegible] Suppl 27 [illegible]

TABLE II The values for prothrombin, factor VII, factor IX, factor X, fibrinolysis, plasminogen and inhibitors of fibrinolysis during the investigation period

		Prothrombin (%)	Factor VII (%)	Factor IX (%)	Factor X (%)	Fibrinolysis (mm²)	Plasminogen (%)	Inhibitors (%)
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	18	116	110	180	112	0	75	95
	23	108	90	165	112	0	67	120
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	10	90	80	165	120	0	60	100
	13	100	90	165	112	0	67	105
	15	100	96	165	104	0	65	85
	17	94	90	165	98	0	—	120

plasma (platelet agglutination, release of platelet factor III and clot retraction).

Proactivator: as determined by the method of Blix (4).

2 Results

Concerning prothrombin, factor VII, factor IX and factor X no significant variations were observed. There were no systematic changes in plasminogen or inhibitors of fibrinolysis and no increased fibrinolytic activity at any time (table II).

The results of platelet and fibrinogen investigations are given in fig 2 and fig 3 shows the concentrations of factor VIII, factor V and proactivator of the fibrinolytic system.

No platelet sensitivity to corticosteroids was revealed.

Discussion

In the present patient most of the criteria of intravascular coagulation has been found, including decreased levels of fibrinogen factor V and factor VIII and a low platelet count The decreased proactivator of the fibrinolytic system is likewise compatible with the defibrination syndrome (4) As is seen in fig 3, there is a remarkable correlation between the proactivator and factor V values Only the prothrombin values were not consistent with intravascular coagulation

In our patient there were two periods of defibrination corresponding to prednisone therapy Between and after these periods, high factor VIII and fibrinogen values indicated a hypercoagulable state in the blood (9) This could have been coincidental, but could indicate that corticosteroids might induce the defibrination syndrome in patients who are in a hypercoagulable state We have found the platelet count to be a sensitive indicator of the defibrination process It was not possible to reveal platelet sensitivity to corticosteroids by in vitro techniques

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More evidence is added to the hypothesis that there is a connection between hemangiomas and the defibrination syndrome In a patient with hemangiosarcoma in the spleen and a defibrination process, the latter might have been intensified by the administration of prednisone

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From Medical Department C (Head P Horstmann, M D) Odense City and County Hospital Odense, Denmark

Mononucleosis Infectiosa, a 5-year Material with Special Reference to the Effect of Prednisolone Treatment

By

Erik Simonsen and Knud Christensen

Since Lopez et al (10) published the results achieved by cortisone treatment of 2 children suffering from monucleosis infectiosa (m i), a number of reports as to the effect of treatment with steroids on larger materials of patients have appeared

Bender et al (2) treated 11 cases of m i with ACTH The patients were getting better clinically within the first 24 hours, and temperature was normalized in a few days

Bernard (5) treated 22 cases of m i with 100 to 200 mg of cortisone a day for 5 days The temperature was normalized within 2 days, and the changes of tonsils, the enlargement of the lymph nodes, and the splenomegaly likewise disappeared quickly, the duration of the disease was reduced

Mason et al (11) treated 17 patients with prednisolone 5 mg 4 times a day for 1 to 3 days and thereafter the dose was scaled down by 5 mg a day All the patients had either distinct changes of their tonsils, enlargement of their lymph nodes, and periedema, or protracted temperature Subjective recovery began during the course of 24 hours The changes of the tonsils, the enlargement of the lymph nodes as well as the periedema disappeared in the course of 2 or 3 days

In a double blind study comprising 9 patients treated with prednisolone and 15 patients treated with aspirin Evans (6) did not find the effect of 30 mg of prednisolone a day to be better than that of aspirin

Antila et al (1) have investigated the effect of ACTH in a large material The doses used were as follows the 1st day 60 i u of corticotropin the 2nd day 40 i u, the 3rd and 4th day 20 i u, and the 5th day 10 i u Fifty one patients were treated with both corticotropin and V-penicillin and 60 patients were treated with V penicillin only In the patients who had had fever for less than 1 week before hospitalization and treatment, the treatment with corticotropin reduced

Submitted for publication May 31 1966

TABLE I Classification by sex and age

	Prednisolone treated patients		Patients not treated with prednisolone	
	Male	Female	Male	Female
0— 9 yrs		2	2	
10—19 —	13	12	18	19
20—29 —	7	4	16	2
30—39 —			3	
≥40		1	1	
Total	20	19	40	21

the duration of their fever, but this did not hold for patients who had had fever for more than 1 week before hospitalization and treatment The toxicity of the disease was reduced with improvement in general condition, but the tonsil changes remained unaffected by this treatment

Material

The present material includes 100 patients hospitalized in the medical department C of Odense Amts og Bys Sygehus during the period December 1959 to December 1964

To warrant our making the diagnosis m i we demanded that 3 of the following 4 criteria be fulfilled

1) A typically clinical progress with tonsillitis fever and universal enlargement of the lymph nodes or a febrile progress with universal enlargement of the lymph nodes

2) More than 60 per cent of lymphocytes plus monocytes in the blood

3) Paul Bunnell s reaction to be positive at a serum dilution of 1 to 64

4) Thymol to be positive i e over 0 13 McLagan units

As the journals lack data from absorption tests centered on Paul Bunnell s reaction we have had no data to guide us in assessing Paul Bunnell s reaction

Four patients fulfil only 2 of the 4 criteria in that for Paul Bunnell s reaction the value was 1 to 32 and thymol was not tested However these patients are included in the material, as they had both typical tonsillitis with yellowish white coats periedema, and universal enlargement of their lymph nodes — and a typical blood picture showing above 60 per cent of lymphocytes and atypical cells of McKinley and Downey s types

Classification by age and sex is given in table I

All patients had the same standard treatment, viz procaine penicillin 300 000 units a day and confinement to their beds until the temperature was normalized and the symptoms had disappeared Thirty nine patients were besides treated with prednisolone 5 mg 3 times a day which accelerated the normalization of temperature All of the 39 patients treated with prednisolone were hospitalized during the last 2 years of the five year period The remaining 61 patients serve as a control material in relation to the effect of the prednisolone treatment

The patients clinical symptoms are shown in table II where the clinical symptoms from some other materials are also stated

Patients with periedema amounted to 28 (28 per cent) of whom 10 were found in the group treated with prednisolone and 18 in the control group Owing to distinct changes of the tonsils and periedema one patient in the group not treated with prednisolone had to be tracheotomised One patient — also in this group — had a peritonsillar abscess

Results

Among 52 patients tested the anti streptolysin titer was found to be increased in 9 cases (18 per cent)

Exanthema was found in 14 cases, but in 6 cases it seemed to be an allergic exanthema, for which reason these are not included in the table Among 8 of this number, the exanthema was in 6

TABLE II Clincial find ngs

	No of pats	Sore t roat (%)	Enlarged cer v cal lymph nodes (%)	Un versally enlarged lymph nodes (%)	Splenomegaly (%)	Icterus (%)	Exanthema (%)
Thomsen (12)	549	97 7	98 3	82 6	42 6	3 8	20 1
Hoagland (9)	200	83	100			8	3
Mason (11)	100	91	95	40	51		12
Own mater al	100	89	90	70	9	9	8
Prednisolone treated	39	95	85	67	3	18	13
No prednisolone treatment	61	85	92	72	8	7	5

TABLE III Laboratory findings

	Prednisolone treated (39 pat ents)	No predn solone treatment (61 pat ents)
> 10 000 leucocytes per µl	46 %	13 %
> 6 000 lymphocytes + monocytes per µl	77 %	69 %
> 60 % lymphocytes + monocytes in the blood	85 %	89 %
Atyp cal cells of McKinley and Downey s types in the blood	89 %	72 %
Paul Bunnell s react on pos tive at dilution 1 64 and more	85 %	77 %
Sedimentation rate < 10 mm/h	8 %	13 %
Sedimentat on rate 10—40 mm/h	74 %	69 %
Sed mentation rate > 40 mm/h	18 %	18 %

cases red, fine spotted and located to truncus and extremities whereas in the remaining 2 cases the exanthema was universal and morbilliform

Three patients — none of whom had lung symptoms — had infiltrates in their lungs Two patients had enlarged hilar lymph nodes

Meningoencephalitis was found in 1 case in the untreated group

The results from differential blood counting Paul Bunnell s reaction and the sedimentation rates in the predni solone treated group and in the non prednisolone treated group are shown in table III

TABLE IV Results of liver function tests + P and − P indicate the prednisolone treated and the untreated patients respectively In the first column the numbers in parenthesis indicate the number of patients with jaundice The average values for the parameters examined are indicated in the column marked x n

	No of examined patients		Patients with increased values		x n	
	+ P	− P	+ P	− P	+ P	− P
Serum bilirubin (standard value < 1 mg %)	11 (7)	13 (4)	64 %	31 %		
Thymol turbidity (standard value < 0 13 units)	39 (7)	43 (4)	87 %	88 %	0 29	0 25
Serum GO transaminase (standard value < 2 units)	20 (6)	18 (3)	90 %	72 %	5 8	3 9
Alkaline phosphatase (standard value < 13 K A *units*)	*10 (7)*	*11 (3)*	*90 %*	*73 %*	*26 8*	*20 4*

TABLE V Liver function tests

	Serum bilirubin		Thymol turbidity		GO transaminase		Alkaline phosphatases	
	No of examined pats	Increased values (%)	No of examined pats	Increased values (%)	No of examined pats	Increased values (%)	No of examined pats	Increased values (%)
Bennike (2)	96	8	99	73			24	33
Gelb (8)	316	20	516	62	54	80	295	64
Futterweit (7)	51	19 6	55	94 5	17	82 3	43	74 4
Own material	24	46	82	88	38	84	21	80

Table IV shows the results from the liver function tests in the prednisolone treated and in the untreated group

Table V shows the results of the liver function tests for all 100 patients compared with the results achieved by other authors

As appears from perusal of the clinical findings and the laboratory tests, there is no difference as to the severity of the disease between the prednisolone treated group and the control group

For estimating whether there is an effect of prednisolone treatment, the material has been divided into 2 groups, viz one group with a duration of disease of 7 days and less before hospitalization and treatment, the other group with a

TABLE VI *Changes of the tonsils at hospitalization*

	Prednisolone treated patients		Patients not treated with prednisolone	
	No of patients	%	No of patients	%
Duration of fever before hospitalization ⩽ 7 days				
–	2	8.0	3	6.8
+	0	0.0	4	9.1
++	13	52.0	19	43.2
+++	10	40.0	18	40.9
Total	25	100.0	44	100.0
Duration of fever before hospitalization > 7 days				
–	0	0.0	2	11.8
–	0	0.0	0	0.0
++	11	78.6	12	70.6
+++	3	21.4	3	17.6
Total	14	100.0	17	100.0

TABLE VII Results of treatment of patients with a duration of fever ⩽ 7 days before hospitalization

	Prednisolone treated (25 patients)		No prednisolone treatment (44 patients)
Duration of sore throat	8.9 days		8.9 days
Duration of the fever after hospitalization	*3.7 days*	$p < 0.01$	5.9 days
Total duration of the fever	8.5 days	$p < 0.05$	9.8 days
Duration of the confinement to bed	12.1 days		9.9 days
Duration of the hospitalization	15.4 days		12.6 days

TABLE VIII Results of treatment of patients with a duration of fever > 7 days before hospitalization

	Prednisolone treated (14 patients)		No prednisolone treatment (17 patients)
Duration of sore throat	7.8 days		9.5 days
Duration of the fever after hospitalization	4.0 days	$p < 0.10$	5.4 days
Total duration of the fever	17.5 days		18.3 days
Duration of the confinement to bed	11.1 days		9.4 days
Duration of the hospitalization	14.5 days		12.8 days

duration of disease of more than 7 days before hospitalization and treatment

In table VI are stated more closely the changes of the tonsils, with a rating of + for flush, enlargement, and coats, thus +++ signifies the presence of all 3 findings

Table VII and table VIII indicate the results achieved at treatment

Discussion

The classification of age and sex as well as the clinical findings correspond to the data of other authors (9, 11, 12)

Among 52 patients examined the antistreptolysin titer was found to be increased in 18 cases, which is in accordance with Bennike's (4) statements

Approximately 80 per cent of the patients suffering from m i had abnormal liver function tests The incidence of increased SGOT alkaline phosphatases, and thymol matches the results obtained by other authors (3, 7 8) As the icteric patients number a relatively large percentage of the patients who had had their serum bilirubin tested, the observed per cent of increased values is relatively high

Among the patients who had fever for less than 7 days before hospitalization the average duration of fever after hospitalization — as far as the prednisolone treated patients are concerned — amounts to 3 7 days, compared with 5 8 days for the control group Accordingly there is found — in the prednisolone treated group — a reduction in the fever period of 38 per cent during the stay in hospital Moreover, the average total duration of fever is shorter among these patients (8 5 days compared with 9 8 days in the control group)

Among the patients who, when hospitalized, have had fever for more than 7 days, a reduction in the fever period is again seen in the prednisolone treated group, but in comparison with the control group the difference is not significant

Changes of the tonsils are not affected by the steroid treatment, which is in agreement with the results achieved by Antila (1) and Evans (6) Contrary to this, Bernard (5) and Mason (11) find quicker regression of the changes of the tonsils when treating with steroids

Both the period of confinement to bed and the period of hospitalization are a little longer in the prednisolone treated group than in the control group This might be due to the fact that — for fear of the increased risk of secondary infections — more caution was displayed in giving these patients permission to get up, just as they were not discharged until the prednisolone treatment had been scaled down completely

Conclusion

The material includes 100 patients with m i The classification of age and sex, the clinical findings, the changes in the blood picture, the occurrence of abnormal liver function tests, and a positive Paul Bunnell's reaction correspond to findings made by other authors

Thirty nine patients have been treated with procaine penicillin and prednisolone, 61 patients with procaine penicillin In patients who have had fever for less than 7 days before commencement of the treatment, steroid treatment can

reduce the average duration of fever, while this is not reduced significantly in patients with fever for more than 7 days before steroid treatment is instituted The changes of the tonsils remain unaffected by the steroid treatment, and the period in which the patients are confined to their beds as well as the period of hospitalization are likewise not reduced through this treatment

As a standard treatment of m i the use of steroids does not seem to be indicated, steroid treatment probably rather ought to be reserved for highly febrile patients in a severe toxic condition as well as for patients with complications

Summary

A five year material of 100 patients suffering from mononucleosis infectiosa has been assembled The clinical findings and the results from laboratory tests are expounded The material in question was reviewed in order to demonstrate a possible effect obtained by prednisolone treatment of m i, 39 patients being treated with prednisolone, and the remaining 61 patients serving as controls It is found that prednisolone gives a significant reduction in the duration of fever among patients who have had fever for less than 1 week before the treatment was instituted However prednisolone treatment of m i was not proved to be curative

It is concluded that steroid treatment ought to be reserved for highly febrile in a severe toxic condition and for patients with complications to the disease

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From the Renal Ward Fourth Department of Medicine, and the Second Department of Pathology University of Helsinki and the Medical Department, Maria Hospital Helsinki Finland

Secondary Amyloidosis

A Study of Clinical and Pathological Findings

By

BORJE KUHLBÄCK and OTTO WEGELIUS

Secondary amyloidosis occurs as a complication in certain chronic conditions, in particular in rheumatoid arthritis bronchiectasis, tuberculosis and osteomyelitis

The pathogenesis of the disease is still obscure Among the numerous theories advanced during the course of years, the most widely accepted seems to be that which is based on the observation of a local cellular activity in the reticulo-endothelium According to Teilum (21, 22, 23) the formation and deposition of amyloid occurs in two stages In the first stage, reticuloendothelial tissue rich in plasma cells shows proliferation and pyroninophilia, probably due to immunological causes. This pyroninophilia is a manifestation of an active RNA system and it is indicative of an increased protein synthesis On transition to the second phase increased PAS positivity occurs in the cells This positivity is attributed to an increased synthesis of glycoproteins which are deposited extracellularly and form the matrix of amyloid It is therefore understandable that in secondary amyloidosis the amyloid substance is encountered in tissues rich in reticular fibres If Teilum's theory concerning the pathogenesis of amyloidosis holds good, it may be assumed that amyloid degeneration will simultaneously start in various organs rich in reticulo endothelium Missmahl s (14, 15, 16 17) investigations in patients with secondary amyloidosis entailing Congo red staining in combination with polarization microscopy of various organs seem to corroborate this assumption

The geographical distribution of amyloidosis has been summarized by Battaglia (3) This condition appears to occur most frequently in Portugal and in Spain Israel and other Mediterranean countries probably as a complication of the so called Mediterranean fever (4, 10, 20) According to the available literature secondary amyloidosis is a relatively rare complication elsewhere

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TABLE I Clinical symptoms and signs in secondary amyloidosis

Primary disease	Signs of liver insufficiency	Intestinal symptoms and signs	Pathologic electrophoresis	Serum cholesterol > 260 mg/100 ml	Congo red retention > 60 per cent	Renal failure	Proteinuria	Renal biopsy performed	Autopsy	Cortisone treatment
1 Lung disease (12 cases)	5	3	11	2	7/9	10	12	5	10	4
2 Rheumatic disease (7 cases)	2	1	5	2	4/5	5	7	1	6	3
3 Bone disease (4 cases)	0	1	4	0	2/2	1	4	3	2	0
4 Malignant disease (3 cases)	0	0	2	0	0/0	3	2	0	3	0
5 Tuberculosis (3 cases)	1	1	2	0	1/1	3	3	0	3	0
6 Miscellaneous (3 cases)	1	1	2	1	1/2	3	3	1	2	0
Total 32 cases	9	7	26	5	15/19	25	31	10	26	7

An extensive study on amyloidosis has recently been published by Christensen (8)

The purpose of the present investigation is to throw light on the frequency of secondary amyloidosis in Finland and to analyse the distribution of amyloid deposits in the body in individual cases Apart from the occurrence of amyloid in various organs special attention was directed to the relationship between renal function and the pathological findings at autopsy, the significance of renal biopsy and the results of cortisone treatment

Material

The series consists of 32 patients with secondary amyloidosis i e all those patients with this diagnosis who were treated in the medical wards of the Maria Hospital during the years 1954—1964 During this time a total of 34,603 patients were treated in this hospital The incidence of amyloidosis was thus 0 09 per cent During the period in question one patient with amyloidosis secondary to myelomatosis was treated in the hospital but since this form of the disease differs from ordinary secondary amyloidosis in regard to pathogenesis and organ distribution this case was not included in the series In 26 cases the diagnosis was established at autopsy in 10 cases it was confirmed by percutaneous renal biopsy

All patients were clinically investigated in regard to symptoms and signs indicative of liver intestinal and renal lesions Apart from the ordinary routine examinations the following determinations were invariably made serum cholesterol Stolte, thymol turbidity test total proteins, serum electrophoresis urine analysis, plasma creatinine, creatinine clearance, analysis of the faeces

Renal biopsy was performed in 10 cases Rectal biopsy was made in only one case, as renal biopsy has been used since 1960 as the

primary diagnostic aid in cases suspected of amyloidosis

The Congo red test was performed in 19 cases Seven patients were given cortisone treatment on account of severe nephrotic syndrome (oedema severe proteinuria elevated cholesterol level, hypoproteinaemia) or as adrenal substitution therapy

At autopsy, performed in 26 cases several internal organs were investigated — although unfortunately, during the nineteen fifties not all important organs were routinely preserved for the purpose of microscopy Furthermore available preparations stained with Congo red as suggested by Missmahl (17) were studied for amyloid in polarized light

The series is shown in table I The patients are classified in six groups which clinically are somewhat heterogeneous The first group (lung disease) includes bronchiectasis pneumonia and pulmonary tuberculosis the second group (rheumatic disease) consists of patients with rheumatoid arhritis, the third group (bone disease) includes osteomyelitis and bone tuberculosis the fourth group (malignant disease) is constituted by hypernephroma and carcinoma of the prostate and bladder the fifth group (tuberculosis of other parts of the body) consists of patients with tuberculosis of the kidneys and lymph nodes and the sixth group (miscellaneous) includes cases of amyloidosis of unknown origin possibly chronic pyelonephritis infection of the gall bladder and/or pleuritis

Results and discussion

The clinical symptoms and signs, certain blood values and other details are shown in table I It is striking how often the cause of the amyloidosis was a disease in those organ systems (lungs joints) which are rich in chondroitin sulphuric acid This has also previously been pointed out (5, 11) On examination of a human amyloidotic liver Schmitz Moormann found that the amyloid contained 0 25 per cent carbohydrate in mucoids containing uronic acid, and 0 66 per cent in mucoids containing neuraminic acid Acid mucopolysaccharides were present in moderately soluble or poorly soluble protein fractions in particular and contained 90 per cent chondroitin sulphate The remainder consisted of hyaluronic acid, chondroitin and heparin (19) The scanty total content of chondroitin sulphate in the amyloid substance need not be regarded as a phenomenon arguing against the suggested correlation between a primary lesion of tissues rich in chondroitin sulphate on the one hand and amyloid degeneration on the other On the contrary these tissues may contain the unknown antigen which precipitates the process

Liver insufficiency intestinal symptoms and elevated serum cholesterol values were not particularly conspicuous in the present series By contrast pathological electrophoretic values, renal insufficiency and proteinuria were observed in the majority of the cases On the basis of these observations it appears that renal function is, perhaps, more sensitive to amyloid deposition than the gastro-intestinal function

The Congo red test was performed in 19 cases Pathological values with over 60 per cent retention were noted in 15 of these Using this limit as a criterion the test thus proved to be of great diagnostic value Two patients developed a severe allergic reaction immediately after injection of the dye stuff In one case the reaction was so severe that it led to grave shock with oliguria and transient impairment of renal function Owing to these experiences the test was

used with a certain cautiousness in the remaining cases Similar reactions have been described by other authors (1)

Seven patients received cortisone therapy In all these cases nephrotic syndrome constituted the indication, in three, the treatment was given on account of the primary disease (rheuma toid arthritis) In none of these cases was a favourable effect of cortisone therapy noted It is difficult to decide whether the amyloidosis was aggravated as a result of the treatment It has been shown experimentally that glucocorticoids aggravate amyloidosis 12, 18) In diseases of the connective tissue, cortisone may be a factor which elicits amyloid degeneration (25) Furthermore, failure to obtain a favourable effect with cortisone therapy in amyloidosis has been reported, and the view has been advanced that cortisone may even be contra indicated (13)

Percutaneous renal biopsy was per formed in 10 cases In all of these it was by this examination that the diagnosis was definitively established As appears from the results of the organ studies shown in table II, amyloid was present in all the 24 renal specimens investigated Hence a great diagnostic value must be accorded to a positive or negative finding on renal biopsy as has been previously pointed out (7, 9) Although a risk of excessive bleeding from an amyloid degenerated tissue exists, no serious haemorrhagic complications oc curred after biopsy A single patient developed a moderate perirenal haematoma, which was resorbed within a few days However, a similar risk of haem orrhage is connected with liver and rectal biopsy also It may therefore be stated that percutaneous renal biopsy is a very important diagnostic aid in amyloidosis and that the procedure is not connected with greater risks than renal biopsy in other diseases, or than liver and rectal biopsy in amyloidosis

Renal venous thrombosis has been described in connection with amyloido sis (2) This complication was not noted in any of our cases

A 46 year old male patient with osteomyelitis and severe secondary amy loidosis (grave proteinuria, hypo proteinaemia with severe hypo albuminaemia and a Congo red retention of 86 per cent) was intensively treated with antibiotics, which resulted in com plete healing of the osteomyelitis On check up six years later no clinical signs of amyloidosis were observed (normal serum proteins, no protein in the urine, a Congo red retention of only 32 per cent), and the patient felt perfectly well and was occupied in heavy work At a further follow-up examination four years later the patient was still in good health, but a renal biopsy specimen exhibited small amounts of amyloid in the glo meruli

Case report

J no 2592/54

Male 46 carpenter As a child he had been healthy In 1943, his right arm was fractured (fracture of the humerus) There was a purulent discharge from the arm for *many* years (osteomyelitis) In 1954, the patient injured his right knee He was admitted to the Maria Hospital, where the knee healed within a month Laboratory examinations revealed the following ESR 130 mm/hr Hb 95 per cent, serum protein 4 4 per cent (alb 12 7 per cent, α_1 glob 8 8 per cent, α 23 per

TABLE II Degree of amyloid deposits in various organs found at autopsy

Pat	No	Heart	Kidney	Liver	Lung	Spleen	Bowel	Thyroid	Hypophysis	Adrenal	Ovary	Prostate	Pancreas	Testis
R S	2990		+	−	−					++			+	
E S	2927		+	±	++	+		++					+	
J N	2761	++	+	+			+		+	−				
E.H	1939		++	+	−	++			±	±	+			
U N	2062	+	+	−		++		+	+				++	
K B	1442		++	+	++	++								
A L	2276		++	+	+	++	++			++				
O R	3004	++	++	+		++			+	++			++	
H P P			++		+		++							
L.M	3154		+	±				+		−				
V T	292	+	+	+	+	++	+	+		++			++	
M L.	112	+	+	+	+	++	++	+	+	++	+		+	
V R	1649		+	+		−								
A L.	1183		+	+		++	+							
V T	1724		+											
K B	1690		+											
A A	817		+	+	+	++	++	+						
O.S	931			±		+	+			++				
A I	206		+	−	+			+		+				
H S	2390				+	++	+	±	+			+		
A S	2099		+		−	+	±							
A G	1371		+	+	−								±	
I T	2368		++	+			++	+	+	+			+	
R T	1713		+											
	328	−	+	±	+	++	+	+	±	+			−	−
	342		++	+	±	++	+	++	++	+	±	±	++	

++ = abundant amyloid deposits
+ = obvious amyloid deposits
± = uncertain amyloid deposits
− = no amyloid deposits

cent β 25 3 per cent γ 30 2 per cent) non protein nitrogen 20 mg/100 ml Congo red retention 86 per cent urinary alb + Esbach 12 per thousand The patient was again examined at the ward two months later and the same changes were noted No renal biospy was performed During these hospital stays the patient was given large doses of antibiotics (penicillin tetracyclin streptomycin)

In 1960 the patient was re admitted to the ward The general condition was this time much better The arm showed complete healing and laboratory examinations gave the following values ESR 28 mm/hr Hb 14 6 g/100 ml serum protein 7 5 per cent (alb 42 7 per cent α_1 glob 7 0 per cent α_2 10 6 per cent β 15 9 per cent γ 23 8 per cent) endogenous creatinine clearance 62 ml/min Congo red retention 32 per cent

TABLE III Amyloidosis in various organs

Organ	Cases positive for amyloid in relation to total no of cases studied
Kidney	24/24
Liver	17/20
Spleen	15/16
Lung	11/15
Bowel	13/13
Pancreas	10/10
Heart	5/6
Thyroid	11/11
Hypophysis	9/9
Adrenal	11/13

urinary alb ±, Esbach — Renal biopsy methyl violet staining revealed metachromatic areas in all glomeruli and in many small arteries and arterioles, indicating moderate amyloid deposits

In 1964, the patient was re admitted for further investigations The general condition was very good and the patient had been occupied in heavy work The following laboratory test values were obtained ESR 27 mm/hr Hb 12 1 g/100 ml serum protein 6 8 per cent (alb 53 7 per cent α_1 glob 5 2 per cent, α_2 9 1 per cent β 14 7 per cent γ 17 3 per cent) plasma creatinine 1 55 mg/100 ml urinary alb — Esbach 0 2 per thousand Renal biopsy moderate changes indicative of amyloidosis

The case described seems to indicate that in occasional cases secondary amyloidosis may be to a certain extent reversible, if complete healing of the primary disease is attained Healing of secondary amyloidosis has also previously been reported in certain cases (24)

During the period of observation, 26 of the present patients died In four of these cases renal biopsy had been performed Autopsy was performed in all these cases

The results of the organ examinations performed on these 26 patients are shown in tables II and III and in figs 1—6 It is striking that with the special staining employed, amyloid was detected in the majority of the organs investigated Thus, amyloid was found in all renal, intestinal, pancreatic, thyroid and pituitary specimens that were examined Furthermore, amyloid was demonstrated in the majority of the investigated specimens from the liver, spleen, lungs, heart and adrenals Briggs, too, observed amyloid in most organs studied (6) It should be mentioned that by examination in polarized light of organs stained with Congo red, even



Fig 1 Five glomeruli filled with amyloid substance Congo-red staining Angular magnification 240 ×

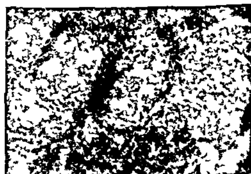


Fig 2 The same area of the kidney as in fig 1 studied in polarized light



Fig 3 Deposition of amyloid in the walls of pancreatic vessels A portion of an island of Langerhans can be seen to the left Congo-red staining Angular magnification 240 ×



Fig 4 The same area of the pancreas as in fig 3 studied in polarized light.



Fig 5 Large amounts of amyloid in the stroma between the follicles of the thyroid Congo-red staining Angular magnification 240 ×



Fig 6 The same area of the thyroid as in fig 5 studied in polarized light

small amounts of amyloid are demonstrable as has been previously emphasized by Blum and Sohar (4) and others

The histological finding in the kidneys, investigated after autopsy, showed a good correlation with the degree of impairment of the renal function (fig 7) Histological changes of the kidneys were invariably observed Furthermore, all patients but one exhibited proteinuria and renal insufficiency was present in all cases

From what has been stated above it may thus be concluded that when secondary amyloidosis occurs, practically all parenchymatous organs are in

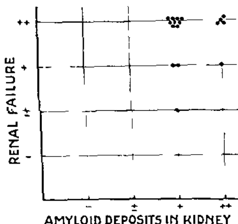


Fig 7 The correlation between amyloid deposits in kidney and renal failure

volved Impairment of the function of these important organs, i e the kidneys, liver, bowel, pancreas, adrenals and pituitary, is therefore to be anticipated in various stages of the disease As a diagnostic aid, renal biopsy in particular, but also rectal and, perhaps, liver biopsy may be recommended There is no therapy available, cortisone treatment has no effect and may even aggravate the condition As appears from the case described in the foregoing, it is important to treat the primary disease radically, if possible, in order to prevent the development of amyloid degeneration Bronchiectasis, bronchitis and osteomyelitis each ought to be given effective and protracted treatment with antibiotics In tuberculosis an active therapy and careful checking of the results are necessary If these measures give no result, and if any signs of amyloidosis occur, radical procedures must be mediated Among such measures mention may be made of lobectomy or pulmectomy in cases of unilateral bronchiectasis or tuberculous foci and amputation of an extremity which is the site of chronic osteomyelitis or tuberculosis If these measures are taken in time the secondary amyloidosis may be reversible If they are too long postponed the disease progresses and the outcome will be fatal within a few years

Summary

A series of 32 patients with secondary amyloidosis was investigated In 10 cases the diagnosis was established by percutaneous renal biopsy in 26 cases it was verified at autopsy The main primary diseases which led to amyloidosis were bronchiectasis, tuberculosis, rheumatoid arthritis and osteomyelitis The Congo red test was performed in 19 cases In 15 of these, pathological values (over 60 per cent retention) were obtained

Seven patients received cortisone treatment In none of these cases was a favourable effect of the therapy observed In the present series renal biopsy proved to be the best diagnostic aid

A case of severe secondary amyloidosis following osteomyelitis is described After active antibiotic treatment the osteomyelitis healed, and at follow up examinations six and ten years later the patient was found to be healthy, but on renal biopsy small amounts of amyloid were still detected

Organ examinations revealed amyloid in all investigated specimens of the kidneys, intestine, pancreas, thyroid and pituitary, and in most specimens of the liver, spleen, lungs, heart and adrenals

The importance of treating the primary disease effectively is emphasized If necessary, radical procedures should be resorted to If the primary disease heals, the amyloidosis may be reversible Otherwise the course is fatal

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From the Cytogenetic Laboratory, Institute of Psychiatry Århus State Hospital, Risskov the State Department of Neurosurgery University of Århus Århus the Male Hypogonadism Study Section University Hospital Copenhagen the Hormone Department Statens Serum institut Copenhagen and the Institute of Medical Genetics Copenhagen Denmark

Psychopathology and Testis Histology in a Patient with the XYY syndrome

By

JOHANNES NIELSEN, ANNE LISE CHRISTENSEN, SVEND G JOHNSEN and ANDERS FROLAND

The present case, a 26 year old single mechanic with 47 chromosomes and sex chromosomes XYY, was found in a combined prevalence incidence study of Klinefelter s syndrome at the Århus State Hospital in which 10 per 1,000 patients have been found to have Klinefelter syndrome with more than one X chromosome in all or part of their cells Very few patients with 47 chromosomes and sex chromosomes XYY have been described None of them have been studied from a psychiatric point of view The sex chromosome constitution XYY might be classified as the XYY syndrome

Previous studies of patients with the XYY syndrome

Sandberg et al (14) and later Hauschka et al (7) were the first to describe a patient with 47 chromosomes XYY He was a 44 year old strongly built man without any serious physical defects and with no signs of hypogonadism He was described as being of average intelligence He earned his living through various manual tasks, but he had difficulties in keeping employers satisfied with his work performance He had been married twice In his first marriage with four childbirths and one spontaneous abortion there was one twin birth, and a daughter with a possible Turner mosaic One of the twins died three days after birth There were two births in the second marriage and one spontaneous abortion One of the children in the second marriage suffered from Down s syndrome

There is no information about the mother s age but the father was 25 years old when the patient was born The mother died in her forties from carcinoma of the breast The only sib-

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lings were two married sisters aged 43 and 36 in good health One sister was childless, and the other had several normal children There were otherwise no illness in the family

Court Brown et al (1) found a 23 year old man with 47 chromosomes, XYY He had talipes equinovarus Penis and scrotum were small, and testes were very small and soft He had scanty pubic hair with male distribution and he had no beard growth He was of low intelligence Maternal age was 19, and paternal age 27 at the time of birth, and the patient was number 1 of 4 siblings

Sandberg et al (15) described a 12 year old boy with 47 chromosomes, XYY Obesity was described as his major problem, and the parents attributed his lack of vigor and ' pep' to the obesity He had kept up with his class in school and was reckoned on testing to be of 'average intelligence

The right testis was in the scrotum and slightly smaller and softer than normal The left testis could not be palpated in the scrotum or in the inguinal canal

The mother was 21 and the father 22 years old when the patient was born The patient had two brothers, 10 and 8 years old The older brother was enuretic and the younger brother had suffered from the same condition until recently There was a family tendency for enuresis on the father s side Both brothers had undescended testes until recently The size and consistency of the testes seemed to be normal in both brothers A first cousin died of leukemia at the age of 3 years

Hayward and Bower (8) described a 4 year old boy with Sturge Weber s syndrome, 47 chromosomes and trisomic 22 which, however, was later identified as being XYY as described by Dent et al (2) This boy was described as showing gross mental retardation Locomotor abilities at the age of 3 years and 10 months corresponded to a 12 month level on the Griffith's scale, while other abilities (personal, social, hearing and speech, eye and hand, and performance) were at a 6 month level His face was not suggestive of mongolism

Maternal age at the time of birth was 32 His mother died shortly after his birth His father and two siblings were healthy, and there was no relevant family history

Milcu et al (11) described a 7 year old boy with 47 chromosomes, XYY He had hypospadia, a small penis, and small descended testicles He was described as being of average intelligence Parental age and family history were not described

Hustinx and van Olphen (9) described a boy with Marfan s syndrome, 47 chromosomes and XYY He was 11 years of age and the first in a sibship of 6 children His father was 37 at the time of birth and his mother 36 His father suffered from Marfan s syndrome and he died suddenly from a ruptured aortic aneurysm or a myocardial infarction Both testes were of normal size and consistency

Two sisters had also Marfan's syndrome

Ricci and Malacarne (13) found a 15 year old boy with 47 chromosomes, XYY He had external genitalia of normal adult type, but he was grossly mentally retarded with an IQ of 59

At the time when he was born his mother was 19 and his father 23 years old. At the age of 20 after being deserted by the father the mother had a depressive reaction and received E.C.T.

Verresen and van den Berghe (19) briefly described a 9 year old boy with mongolism, 48 chromosomes, trisomic 21, and XYY. The question of hypogonadism could not be decided, and no information is given about family history or parental age.

Fraccaro et al. (4) described two mentally subnormal children 2 and 8 years of age respectively with 47 chromosomes, XYY. One of these children had frequently minor fits, an undescended right testis, and deficiency of the ventral aspect of the prepuce. The other boy was grossly mentally defective, and his testicles were not descended. Maternal age for the two cases was 39 and 43 and paternal age 40 and 47, respectively.

Uchida et al. (18) reinvestigated a patient described by Dunn et al. (3) as 47, trisomic 22, and found that it was actually a case of 47 chromosomes, XYY.

Townes et al. (17) found a 5 year old boy with 48 chromosomes, XYYY. He was described as being somewhat retarded in psychomotor development. He walked at the age of 21 months and spoke his first words at the age of 2 years. IQ was 80. The genitalia were unremarkable. The patient's mother was 24 and his father 37 at the time of birth. The family history was negative except for a female first cousin with trisomic 21 mosaic and Down's syndrome.

Jacobs et al. (10) made a survey of 197 mentally subnormal male patients with dangerous violent or criminal propensities in an institution where they were treated under conditions of special security. Seven of the 197 patients had 47 chromosomes and sex chromosomes XYY, one had 48 chromosomes and sex chromosomes XXYY, and one had an XY/XYY mosaic. There were thus 3.5 per cent of the 197 patients with the XYY chromosome complement. Three

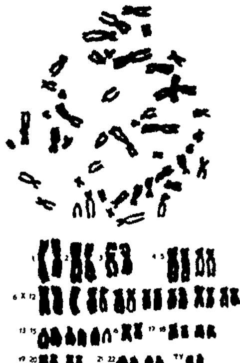


Fig. 1. Karyotype with 47, XYY. Appr. 2 000 times.



Fig. 2. Chromosome groups 19—20, 21—22 and YY from 4 karyotypes with 47, XYY. Appr. 2 000 times.

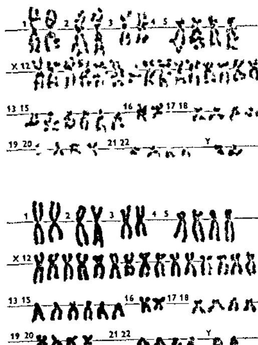


Fig 3 Above autoradiogram of the chromosomes of a cell from a blood culture incubated with tritiated thymidine Below after removal of the silver grains Appr 2 000 times

patients had structural anomalies of the autosomes None of the patients are described from a psychiatric point of view

Methods and results

Sex chromatin and chromosomes

Feulgen stained buccal smear was chromatin negative Chromosome analysis on leucocyte cultures according to the method described by Moorhead et al (12) slightly modified showed a modal figure of 47 and sex chromosomes XYY as shown in figs 1 and 2

Fourty seven metaphases were counted and analysed All 47 metaphases had sex chromosomes XYY 4 lacked a chromosome in different autosome groups 1 lacked a chromosome in the 6 X 12 group as well as in the 13—15 group but all 47 metaphases counted and analysed had 6 small acrocentric chro-



Fig 4 Th... ...omes ... small acrocentrics ... with tritiated th... 2 000 times


a


b


c


d

Fig 5 Testicular biopsy from the case of XYY complement Small tubules thick basal membranes poor tubular content degenerative changes in spermatocytes and spermatides moderate diffuse Leydig cell hyperplasia a) 25 × b) 100 × c) 250 × d) 400 × Stain iron hematoxylin acid fuchsin, ponceau de xylidine fast green (HFPG)

mosomes 2 of which were clearly Y chromosomes as is shown in fig 1 and 2

Skin culture sex-chromatin investigation of testicular tissue and autoradiographic studies were made by one of us (A F) Feulgen stained sections of testicular tissue revealed chromatin negative Leydig and Sertoli cells Chromosome studies on skin cultures were made according to Froland (5) 30 cells were counted 28 had 47 chromosomes The additional element was undoubtedly a Y chromosome so these cells had probably also an XYY sex chromosome complement

Autoradiographic studies

Autoradiographic studies were carried out as indicated by Froland (6) Suitably labeled cells were photographed and after removal of the silver grains the cells were relocated and the small acrocentrics including the Y chromosomes determined morphologically in the microscope The corresponding chromosomes on the photographs were noted and cut out

In fig 3 the karyotype of the patient is shown with labelling of the chromosomes and after removal of the grains from the same cell In fig 4 the labeled Y chromosomes and the small acrocentrics from 12 cells are shown

It will be seen that the Y chromosomes are rather heavily marked in comparison with the other chromosomes especially nos 21 and 22

Hormone excretion

Urinary excretion of follicle stimulating hormone was 60 m u per 24 hours (normal

range for that age 6—75 m u with a mean of 30 m u per 24 hours) urinary excretion of 17 ketosteroids was 11 6 mg per 24 hours (normal range 10—30 mg per 24 hours) The distribution of the different fractions of the 17 ketosteroids as normal th an androgenic fraction of 3 3 mg per 24 hours (normal range 2 9 to 8 0 mg per 24 hours)

Testis histology

The testicular biopsy was studied by one of us (S G J)

The biopsy specimen was fixed in Stieve s solution double embedded in collodion paraffin and sectioned at 6 μ Sections ere stained with iron hematoxylin differentiated in picric acid double counterstained in acid fuchsin and ponceau de xylidin further differentiated in phosphomolybdic d and finally counterstained in fast gr n FCF (HFPG stain)

The biopsy (cf fig 5 a— o s that tubuli are uniformly reduced in Mean tubular diameter is 136 μ l alue 170—250 μ) Tortuousity s g ea du ed Basal membranes a e thickened n ome tubules hyalinized Ho eve no h e found complete tubula hyalin at S ne of the tubules con a n only Se l The rest contains a reduced numbe o g al cells The number of spermatogonia la ly normal Sperma oc es are hea l d ed and display degene at e phenomena p chromatin s ru u e Fe spermatid e fo nd and onl a tubules contain a all number of spe zo c nucle most o h m be n abnor haped Leydig cells sl o a moderate e t e hyperplas a Kl ne felter fea u e a hyalinized areas and clumping of l g ls are totally absent Ma n fea r ps ncomplete puberal developmen

Physical examination

Somatic exa n n ho s a masculine looking normall b nan 182 cm tall and weighing 6 k he gained 12 5 kg during a 7 week s he mental hospital His chin is some h d ng but th n normal l m ts Bea h axillary ha r growth chest ha r g a d ha r growth on arms and legs are normal Pubic hair gro th s scanty but with a masculine distribution There is no gynaecomastia The extremities are of normal length and the muscles normally developed Penis and scrot m are of normal size but testes are smaller and more soft than normally The right testis s approximately 3 cm and the left 4 cm from pole to pole There s an atheromata on the scrotum measuring approximately one cm in diameter which is removed at the same time as testis biopsy is taken Further somatic examination including neurological examination shows normal conditions Colour vision s normal ECG is normal

EEG shows a dominant activity of some hat slow alfa rhytm There are no abnormal potentials no side difference or focal changes Hyperventilation provoces scattered 5—6 Hertz potentials Conclusion no definite abnormality ESR s 4 mm per hour Hb 15 9 g % X ray of spine skull elbows arms hands lungs and heart shows normal conditions There are no signs of osteoporosis

Psychiatric and psychological examination

The father who s a labourer was 26 years old when the patient was born at the age of 59 he s healthy and still working The mother was 24 years old when the patient was born She s healthy physically as well as mentally The patient s number 2 of 4 siblings he has 3 sisters who are healthy

There s no mental illness or inheritable illness of any kind in the family The patient developed normally as a child He had good relations with his parents and he grew up in a harmonious and good home He left school after the compulsory seven years and he did rather well at school When he as a boy he played with boys and girls like any other boy at his age He as ho ever some hat shy till the age of 16 mainly because of nocturnal enuresis — a disorder which bothered him quite a lot From the age of 16 to 20 he was an apprentice in a shipyard where his father worked as a labourer and later as a controller He qualified as a mechanic at the age of 20

From the age of 20 he started drinking and it seems that he has been drinking mainly because of desire for company shyness, and difficulties in getting into contact with girls. He never seems to have become a real addictive alcoholic Since the age of 20 he never kept a job for more than a few months except when he was working as a ma e in ships going to North as well as South-America. He has made two suicidal attempts, at the ages of 24 and 26 respectively.

At the age of 26 he is admitted to the Århus State Hospital eager to be relieved from the following symptoms which have been increasing in intensity during approximately four years He is afraid of crossing the streets and he has difficulty in deciding when to cross He has the feeling that people are looking at him, and laughing at him in the street and in restaurants He has feelings of insufficiency and inferiority He has a compulsive desire to look through coat pockets in restaurant-cloakrooms when he is drunk and to take any coins he finds in the pockets. He has also been afraid of a desire to join a burglar gang

Puberty seems to have occurred around the age of 16 He began masturbating at the age of 12 and he is still masturbating nearly every day He had his first relation with a girl at the age of 15 and he tells that he has had several sexual relations with girls since that time

Psychological testing

The patient s attitude in the test situation reflects immaturity and a general ego weakness He is asking for help and avoiding difficult situations. Emotionally he is labile with only little control of impulses and thoughts

On the WAIS he achieves a full scale I Q of 93 on the verbal tests the I Q is 99 and on the performance tests it is 86 The inter test scatter is rather high information being the best solved test with a scaled score of 12 and digit symbol and block design the poorest with scaled scores of 5 Digit span gives a scaled score of 7 and he is only able to repeat 3 digits backwards

He works slowly and handles the test problems in a disengaged and passive way His thinking is vague self-centered, and with a tendency to concreteness

The projective tests (Rorschach word association test T.A.T) indica e primitive impulses and conflicts of identification and sexual role Content analysis reveals polymorphous libidinal fantasies. The patient s imageries are centered around sexuality homosexual manifes ations, and other perversions Besides the fantasies there is a marked ambivalence with respect to sexual role showing deep-rooted identification problems The emotional tone is rather sad

The defence mechanisms are repression, denial, and to a certain extent projection The defences are so weak that the deep-rooted identification conflicts the drives and the infantile sexual fantasies pour out into the patient s conscious thinking and influence his behaviour Consequently he cannot be described as a true neurotic patient nor do the tests give evidence of psychosis since the reality testing is within the limits of normal functioning

Stay in the State Hosp tal

During the seven-week stay in the Århus State Hospital he is treated with antabus and chlorprotixene work therapy and supportive psychotherapy He feels gradually more stable of mood, the anxiety disappears, and he has no fear any longer of walking in the streets as before admission He tells that he actually feels much better not drinking, and he gains 12 5 kg in weight during the 7 weeks During admission he is very talkative with a great need of contact and support He appears somewhat immature and unrealistic and he seems to cover up for his weak points and over-compensate by boasting for instance of his potency His description of great libido appears however to be true There are no psychotic symptoms and, except for anxiety no real neurotic symptoms

Discussion

As far as intelligence is concerned there is a rather great discrepancy between

the performance I Q of 86 and the verbal I Q of 99, a discrepancy which is found in Turner's syndrome but usually not in Klinefelter's syndrome and normal men without any organic reduction The inter test scatter is high as found in Klinefelter s syndrome In the psychological test it appears that his intellectual function is repressed by his conflicts In patients with Klinefelter s syndrome it has also been found that the intellectual performance is repressed, but more by immaturity and lack of initiative than by conflicts Of the ten previous scattered cases with 47 chromosomes, XYY, three were described as being of average intelligence, two of low intelligence, and four grossly mentally retarded In two cases the intelligence is not described The only patient with 48 chromosomes and XYYY was described as having an I Q of 80 The seven cases of XYY recently reported by Jacobs et al (10) were found among mentally retarded patients In none of the cases described is there any report of a more thorough psychological testing and psychiatric examination It is thus impossible to make comparisons, or to predict anything about intelligence in patients with 47 chromosomes XYY from experience with one such case but the finding of 3 5 % patients with XYY among 197 mentally retarded, dangerous, violent criminal patients may indicate that there is some correlation between low intelligence and XYY

Sexual life

Our patient appears completely different from patients with 47 chromosomes, XXY in having an abnormally strong libido which he tells about himself and which appears in the psychological test to an extent never seen by an experienced psychologist in normal men with 46 chromosomes and sex chromosomes XY There seems, however, to be a discrepancy between his libido and his potency, so that he has an abnormally low potency and an abnormally high libido — which, together with identification problems, actually might be one of the main causes of his conflicts as described in the case history and the psychological test It may be this discrepancy that is the cause of his unrestrained desire to steal money from coat pockets While he was in the hospital a strong libido was released by smashing a window The sexual problems have not been described in any of he previously reported cases of XYY and comparison is thus not possible

It seems worth while to look for more patients with XYY among psychiatric patients with such a discrepancy between libido and potency and with conflicts as our patient — such patients may mainly be diagnosed as psychopaths or as patients with character disorders — and especially among such patients who have a criminal record for violence A chromosome screening of patients with such disorders is planned by one of the authors (J N) in order to look for more cases with the sex chromosome complement of XYY It is too early to draw any conclusions, but it may be that the double Y tends to give an increased libido but a decreased potency

Criminality

One of the reasons for admission of the present patient was that he was afraid of an urge to become criminal and he could not resist pocket picking in cloak rooms when he was intoxicated

Jacobs et al (10) found seven patients with XYY among 197 mentally retarded, dangerous violent criminal patients There might thus be a correlation between the sex chromosome constitution of XYY and criminality, but as mentioned by Jacobs et al it is not clear whether the high frequency of XYY patients found among mentally retarded patients with dangerous, violent criminal propensities is related to aggressive behaviour or to mental deficiency or to a combination of both factors

Our patient was not mentally retarded nor was his criminality of a violent or dangerous character

It would be of interest to study the relation between sexual conflict in patients with XYY sex chromosomes and their criminality

Personality

The causes of the patient s development of sexual conflicts and anxiety symptoms do not seem to be found in his childhood It seems most possible that the XYY complement is the cause of a faulty personality development and discrepancy between libido and potency difficulties in contact with girls dysphoric periods self referring tendencies feelings of insufficiency anxiety and inferiority with consequent symptomatic alcohol abuse and suicidal attempts Many traits in our patient s personality development are similar to those seen in patients with Klinefelter s syndrome who also often react with depressions suicidal attempts and symptomatic alcohol abuse, but patients with Klinefelter s syndrome appear to be more immature with low libido as well as low potency

Somatic

Our case was discovered because of rather small and soft testes which were however, much bigger than the testes of patients with chromatin positive as well as chromatin negative Klinefelter s syndrome Apart from the rather small testes and somewhat scanty pubic hair growth there were no hypogonadal traits, and as such patients are chromatin negative they are not easily found by clinical examination

Of the previously reported cases with XYY complement one was married and had children two had testicles of normal size, five were described as hypogonadal and in ten patients the question of hypogonadism is not dealt with

Our patient was 182 cm tall Jacobs et al (10) found that 6 of the 7 patients with XYY found among 197 mentally retarded criminal patients were more than 180 cm tall They actually calculated the risk of having an XYY chromosome complement to be approximately 1 2 in patients more than 180 cm tall in the special hospital population they studied at "Carstairs State Hospital in Scotland This finding seems to indicate a certain correlation between height mental retardation and a double Y chromosome complement

Aetiology

The XYY karyotype may be produced in two ways. Non disjunction may take place during spermatogenesis resulting in a YY sperm, or a normal XY zygote may be subject to mitotic non disjunction producing an XO and an XYY cell. The XO cells might prove less viable. No possibility exists at present to decide between these two types of origin.

Autoradiography

The results indicate that Y chromosomes replicate late. This has been shown already by other authors (16). It might have been expected that two Y chromosomes in the same cell would not behave in the same way with regard to DNA synthesis. The pictures clearly show that no difference is present in the two Y chromosomes.

Testis histology

The histology of the testes in patients with XYY complement has not been described previously. It is interesting to note that the microscopic picture is widely different from that in Klinefelter's syndrome (XXY). Whereas, in the latter, the testicular tubules are mostly completely hyalinized and Leydig cells are present as large masses, the tissue is better preserved in the XYY-condition. Our patient displayed a uniform picture of poor puberal testicular maturation with moderate degenerative processes, from this picture it is clear that the pathogenesis of hypogonadism is different from that in Klinefelter's syndrome. In a large percentage of the patients with XYY complement described, testicular retention or testicular hypoplasia was noted (Dunn et al (3), Fraccaro et al (4) (two cases), Court Brown et al (1), Sandberg et al (15), Vignetti et al (20)). This indicates that the presence of two Y chromosomes might interfere with the hormone production of the foetal Leydig cells and thus with the male sexual differentiation. The testicular biopsy in our patient is consistent with such changes. The fact that the patient in adult age excreted normal amounts of androgen metabolites does not invalidate this probability. Studies on the testicular function in more cases are, however, needed in order to elucidate the relationship between the XYY chromosome complement and the gonadal development. The question whether the psychic and somatic changes are linked together or are independent symptoms must await further studies in XYY patients.

Summary

A 26 year old man with 47 chromosomes, sex chromosomes XYY and rather small testes has been studied from a cytogenetic, psychiatric, psychological, and testis histological point of view.

Except for a strong libido the psychopathological study showed several similarities with what have been found in patients with Klinefelter's syndrome, as far as testis histology is concerned, there was poor puberal testicular maturation with moderate degenerative processes — a picture quite different from that in Klinefelter's syndrome. Autoradiography showed the two Y chromosomes rather

heavily and equally marked, indicating that they behave in the same way with regard to DNA synthesis

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From the Department of Medicine (Head Nils Tornblom M D), Umeå University Umeå Sweden

Studies of "Auto-immune" Reactions in Adult Patients with Chronic Kidney Disease

By

OLLE LARSSON

It has been assumed for a long time that immune reactions may influence the development of the different forms of glomerulonephritis, more so than in other kidney diseases Many observations support the hypothesis that auto-immunity may be of importance in this development

A disease pattern similar to that found in man can be produced in animals treated with specific "nephrotoxic" antisera (31) Later on this nephrotoxic nephritis can be transferred to another animal by parabiosis or by injection of peripheral white blood corpuscles (30) In some experiments it has been possible to produce a true auto-immune nephrosis by immunization with the animal's own kidney tissue in Freund's adjuvans (13) This disease can also be transferred by the injection of lymph node cells from the sick animal to a healthy one (12)

In man subnormal values of serum complement during the development of acute and sub acute glomerulonephritis may indicate the presence of immune reactions (20, 34) Many investigators have found circulating anti kidney antibodies in patients with glomuleronephritis (5, 17, 19, 21, 29) However, the true connection between these auto-antibodies and the actual disease is uncertain The methods have been criticized and it has not always been possible to reproduce the results (6, 11) In 1956 Wagner and Prokop (36) tested patients with diffuse glomuleronephritis by intracutaneous injections with a saline extract from human kidneys Positive findings might have indicated the presence of cell bound immunity but all reactions were negative

Using the fluorescent antibody technique, Mellors and Ortega (24) were able to show that in glomerulonephritis gamma globulin was present in the thickened basement membrane in the glomeruli This finding together with the presence of an in vivo bound complement with the same localization has repeatedly been confirmed (9 16 18)

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TABLE I Total number of tests performed

Diagnosis	IC-test		PHA test		FA test	
	Total	Positive	Total	Positive	Total	Positive
Glomerulonephritis	18	2	5	1	5	0
Pyelonephritis	17	4	5	2	5	0
Diabetes mellitus	14	2	5	0	5	0
Hypertension	13	2	0		0	
SLE	10	2	9	1	9	[1]0
Other collagen disease	13	0	4	1	4	0
Other	33	2	5	1	5	0
Total	117	14	33	6	33	0

[1] Nuclear fluorescence only
IC-test = intracutaneous test with human kidney antigen
PHA test passive haemagglutination test with human kidney antigen
FA test = fluorescent antibody test with human kidney antigen

and strongly supports the assumption that immune reactions influence the pathogenesis

It is also of great interest that tissue-bound gamma globulin and complement are present in the glomeruli in two other chronic kidney diseases, namely the lupoid nephritis of systemic lupus erythemathosus and the nephropathy of diabetes mellitus (2, 16, 18, 25 26 28) Bloodworth states that the changes seen in the kidneys during the course of glomerulonephritis systemic lupus erythemathosus and diabetes mellitus can appear morphologically identical both by light microscopy and by electron microscopy (3)

The histological picture seen when a transplanted kidney is rejected bears some resemblance to that of chronic pyelonephritis, and the cooperation of immunological factors in certain cases of this disease has been discussed (32) However, neither tissue bound gamma globulin nor circulating anti kidney antibodies have been demonstrated here (14)

The purpose of this investigation has been to test the theory that intracutaneous injection of human kidney antigen can be used to demonstrate any auto-immune reactions in patients with chronic damage of the kidney Simultaneously, the presence of circulating anti-kidney antibodies has been investigated with the help of passive haemagglutination and of a fluorescent antibody technique

Material and methods

Patient material

All patients were observed at the Medical Clinic, Umeå Skin tests on 117 adult patients and sera from 33 of these were tested for the presence of anti kidney antibodies Table I shows the total number of patients The diagnoses were made with the help of generally accepted clinical criteria In some cases kidney biopsies were made

1 Glomerulonephritis Two patients had acute glomuleronephritis and all the others were in various stages of chronic disease

2 Pyelonephritis In all cases signs of chronic kidney damage were present

3 Diabetes mellitus Clinical signs of nephropathy were present in 11 cases

4 Hypertension Kidney damage was diagnosed in 10 patients, but there was no case in which any other form of primary kidney disease was evident

5 Systemic lupus erythematosus (SLE) All patients were treated with cortisone In all of these intermittent proteinuria and pathological sediment were present but the kidney functions were well preserved

6 Other collagen disease The group consisted of 1 patient each with discoid lupus auto-immune haemolytic anaemia chronic hepatitis dermatomyositis 2 with panarteritis nodosa and 7 patients with rheumatoid arthritis

7 Other patients This group consisted of 1 patient each with primary amyloidosis Henoch Schonlein purpura pulmonary tuberculosis chronic pancreatitis cancer coli cancer hepatis and Hodgkin s disease 2 with essential ' haematuria, tuberculosis of the kidney sideropenic anaemia, 3 patients with epidemic nephropathy colitis ulcerosa and lymphatic leukaemia respectively 4 with asthma bronchiale and 5 healthy individuals

Patient sera

All sera were preserved at —20 C Prior to the passive haemagglutination test they were inactivated at + 56 C for 30 min and twice absorbed with fresh sheep erythrocytes

Kidney antigen

The starting material was foetal human kidneys These were collected and treated under sterile conditions after obstetric operations The lengths of the foetuses from crown to heel varied between 10 and 25 cm From 6 to 10 pairs of kidneys were treated together on each occasion The material was kept at a temperature of —30° C The kidneys were cut into small segments, washed free from blood and homogenized with 4 volumes of saline The material was allowed to stand for 24 hours at + 4° C and was then centrifuged The supernatant — the saline kidney extract — was kept under refrigeration in ampoules of 0 5 ml size

The kidney sediment was homogenized again with 5 volumes of saline to form the saline kidney suspension and then kept under refrigeration in the same way as the kidney extract A bacteriological check on the final preparations showed no growth

The total protein content in the different batches of kidney extract varied between 30—40 mg%

The intracutaneous test (IC test)

0 1 ml kidney extract and suspension was injected intracutaneously into the volar aspect of both forearms respectively The points at which the injections were made were checked within periods of 30 min 8 24 and 48 hours or longer All reactions less than 10 × 10 mm were considered negative

The passive haemagglutination test (PHA test)

Fresh sheep erythrocytes were treated with formalin and tannin according to Wide and Gemzell (38) One volume of a 3 3 % erythrocyte suspension was sensitized for one hour at + 37 C with one volume of the kidney extract diluted 10 to 20 times in buffered saline pH 6 4 The treated erythrocytes were washed and used as a 2 5 % suspension in buffered saline pH 7 2 to which was added 1 % normal rabbit serum

Inactivated and absorbed patient sera were diluted ten fold in the same 1 % serum saline solution and serial dilutions were made in small test tubes with a final volume of 0 5 ml in each test tube One drop of sensitized sheep erythrocytes was added to each tube which was shaken and then kept at room temperature The reactions were read after 2 and 12 hours respectively Double tests were made each time as well as simultaneous serial tests with non sensitized sheep erythrocytes All doubtful or positive results were repeated The highest serum dilution

TABLE II Patients with positive reactions in any of the tests performed

Pat no	Diagnosis	Sex	Age	Uremia	IC-test: Kidney extract	IC-test: Kidney suspension	PHA test Titre
1	Chronic glomerulonephr	♂	37	−	−	+	−
2	Chronic glomerulonephr	♂	56	+	+	−	0
3	Chronic glomerulonephr	♀	22	+	−	−	320
4	Chronic pyelonephritis	♀	50	−	+	+	0
5	Chronic pyelonephritis	♀	43	−	+	+	40
6	Chronic pyelonephritis	♀	59	−	+	−	160
7	Chronic pyelonephritis	♀	18	−	−	+	0
8	Diabetes mellitus	♀	55	−	−	+	0
9	Diabetes mellitus	♀	40	−	+	−	0
10	Hypertension	♂	67	+	−	+	0
11	Hypertension	♀	65	−	+	+	0
12	SLE		33	−	+	−	−
13	SLE		46	−	+	+	−
14	SLE	♀	26	−	−	−	80
15	Rheumatoid arthritis		35	−	−	−	80
16	Toxicosis gravidarum		32	+	−	−	40
17	Colitis ulcerosa		16	−	−	+	0
18	Asthma bronchiale	♂	68	−	+	−	0

+ = positive reaction
− = negative reaction
0 no test performed
IC-test = intracutaneous test
PHA test = passive haemagglutination test

which gave a distinct positive agglutination was considered as the final antibody titre

Fluorescent antibody technique (FA technique)

The indirect method was used 23) Sections from human kidney — foetal or adult — were cut in a cryostat fixed in dry acetone and exposed to a few drops of patient serum After thirty min the slides were washed in buffered saline pH 7 2 The presence of bound antibodies (7S gamma globulin) was tested for with the aid of rabbit anti human 7S-serum, conjugated with fluorescin isothiocyanate (Serum FS364 Statens Bakteriologiska Laboratorium Stockholm) The serum was absorbed with tissue powder to eliminate non specific staining

Results

Table I shows the total number of investigated patients and the results of the investigation Table II represents those patients who reacted positively to any of the tests

The IC-test

A positive skin reaction was registered in 14 of 117 investigated patients

The positive reaction consisted of a generally rather small central induration surrounded by an erythema 10 × 10 mm was arbitrarily chosen as the lower

limit for positive reaction Below this limit minor erythema was seen in 16 patients Only one case (patient no 14, table II) developed a rather strong reaction, 35 × 35 mm There was no immediate type reaction in any of the cases Generally the change appeared after a few hours In 9 cases it was maximal after 6 — 8 hours and in the remaining after 24 hours The reaction persisted for 24—48 hours, it was quite indolent, without itching pustulation, necrosis or bleeding

Kidney extrakt and suspension gave the same sort of reaction Four patients reacted positively to both types of injection, 10 to only one

Among the positive cases definite kidney damage could be demonstrated in 12 patients while one patient suffered from asthma bronchiale, the other from ulcerous colitis Two patients had uraemia As can be seen from the tables, there is no demonstrable connection between the result of the test and the underlying disease or the clinical activity of the disease In most positive cases the disease was rather quiescent when the tests were performed

The PHA test

Sera from 33 patients were investigated, and agglutination was recorded in 6 The titres varied between 40 and 320

As for the IC test, the reactions were few and scattered among the different diagnoses

Only in two cases were both IC and PHA tests positive in the same patient Thus the assumption that the positive reaction to the IC-test might be caused by circulating anti kidney antibodies is not supported by the investigation

The FA test

The PHA- and FA test were performed with the same sera In no case was any fluorescence seen which indicated specific binding of gamma globulin to kidney tissue Nuclear fluorescence, not organ specific in character, was seen only in SLE The presence of circulating anti kidney antibodies could not be demonstrated by means of this technique

Discussion

Circulating antikidney antibodies

It has never been possible to identify more specifically those hypothetical kidney antigens in human disease against which the antibodies might be directed It is therefore natural that investigations in which kidney extracts have been used as antigens must be uncertain and difficult to interpret

The kidneys have been collected at different times post mortem from patients varying in age and disease Usually saline organ extracts have been used sometimes after pre treatment with trypsin The sensitivity of the different precipitation agglutination, complement binding and consumption tests varies widely Also in those investigations where the techniques have been similar, the results differ Liu and McCroy (21) used sensitized tannin treated sheep erythrocytes and found positive reactions not only in a high percentage of patients with acute nephritis and nephrotic syndromes but also in several cases of acute

infectious diseases and rheumatic fever Goodman (11) investigated with the same method sera from 20 nephrotic patients Only one weakly positive reaction was seen in a case of SLE Kramer et al (17) used sensitized latex particles and got 15 positive reactions among 36 patients with glomerulonephritis, whereas the test was negative in all other investigated diseases

In the actual investigation a few positive reactions have been registered in glomerulonephritis, pyelonephritis SLE, rheumatoid arthritis and toxicosis gravidarum Thus some form of kidney damage has been present in most of the positive cases, but the results do not show any predilection for any specific disease or the clinical state of the disease

The FA technique is less sensitive than the PHA test In the FA technique however, it is possible histologically to localize the site of reaction between circulating antibody and tissue bound antigen

Neither Cruishank (6) nor Freedman (8) were in any cases of glomerulonephritis able to find any specific binding between circulating gamma globulin and human kidney tissue when the FA-technique was used Both investigators could elute tissue bound serum proteins from kidneys with glomerulonephritis Later on, when this protein was brought into contact with sections from the same or other kidneys no specific binding to the tissue was found Only in SLE could it be shown that the eluted protein reacted for the second time with nuclear substances

The findings of the actual investigation are in all respects consistent with these earlier observations no organ specific binding of circulating gamma globulin to human kidneys has been demonstrated When this technique is used all insoluble kidney antigens are brought into contact with patient sera The negative results do not support the validity of the hypothesis that detectable amounts of anti kidney antibodies are present in the serum of those groups of patients that have been investigated

The IC test with human kidney antigen

In order to get the broadest antigen spectrum possible, both saline extracts and suspensions of human kidney were used However, only foetal kidneys were considered suitable for this investigation Even if the basement membrane in the glomeruli has already partially developed at 2 months (35), it is probable that, from an antigenic point of view, this material is not representative of an adult kidney

Earlier skin tests with homologous organ extracts have been performed in diseases where auto-immune reactions were supposed to have been present (1, 4, 10, 22, 27, 33, 36, 37) There is generally closer agreement between the presence of a positive IC test and pathological changes in the actual organ than between the presence of diagnosed circulating auto-antibodies and these organic changes A positive IC-test has been interpreted both as conditioned by a delayed type hypersensitivity and as a local Arthus phenomenon

The general appearance of the skin reactions observed in different investigations is very similar and in agreement with the reaction described here

Just as in the case of the PHA tests the positive results in the IC test are few and mostly weak No positive reactions were recorded by Wagner and Prokop (36) in their cases of glomerulonephritis In the actual investigation the results were recorded not only in cases of glomerulonephritis but also in other diseases In the majority of these, however, there was chronic damage to the kidneys

The constantly negative FA test and the poor agreement between the PHA and IC tests do not support the assumption that a positive skin reaction is caused by circulating antibodies Delayed type hypersensitivity cannot, however, be excluded

Conclusions

As long as a pathogenic kidney antigen cannot be defined, the problems involved in interpreting the results of the PHA-test are great The negative findings with the FA technique strongly argue against the presence of any specific kidney antigen of aetiological importance in the patient groups investigated The presence of bound gamma globulins and complement in the basement membrane of the glomeruli does not mean that the antigen must be primarily localized in the basement membrane (7) The non specific distribution of positive skin reactions in so many disease groups could be explained if for example, similar antigenic determinants are present both endogenously in the kidneys and exogenously in microorganisms (15) Then the positive reactions might be secondary to i a previous bacterial infections

Summary

In sera from 33 patients, most of whom showed signs of chronic kidney damage of different aetiologies, the presence of circulating anti kidney antibodies has been investigated Six positive sera were recorded, when use was made of passive haemagglutination with formalin tannin treated sheep erythrocytes, sensitized with a saline extract from human foetal kidneys When the sera were tested using the fluorescent antibody technique no positive binding of gamma globulin to kidney tissue was seen

On 117 patients skin tests with saline kidney extracts and suspensions were performed Positive reactions were recorded in 14 patients

The significance of these results is discussed

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From the Institute for Experimental Medical Research (Head F Kiil M D) University of Oslo and the Medical Department VII (Head E Blegen M D Ullevål Hospital Oslo Norway

Plasma Lipids on a Moderately Low-fat, High-carbohydrate Diet, Rich in Polyunsaturated Fatty Acids

By

KNUT KIRKEBY

The study of the etiology and pathogenesis of atherosclerosis has revealed a number of factors hereditary as well as environmental, which may be of importance Interest has been focused on lipid metabolism Disturbances in the serum concentrations of lipoprotein particles and their lipid components, cholesterol phospholipids and triglycerides have been demonstrated in patients with atherosclerotic disease Based on epidemiological and experimental evidence, speculations regarding the possible dietary origin of the disturbances have been made A widespread belief is that a lowering of fat intake and a substitution of polyunsaturated for saturated fats would be preferable in the western diet The evidence for the lowering effect of such dietary changes on the serum levels of cholesterol and certain beta lipoprotein classes is good However it has been shown by a number of investigators that a low fat diet leads to an increase in the concentration of lipoprotein particles of the lowest density (S_f 20--400) and of their main lipid component, the triglycerides (1 6 11 12, 17, 22) The triglyceride raising effect of a low fat diet may not occur when the diet is also low caloric (17) but the evidence on this point is conflicting (15) It has been proposed that the effect is caused by the high carbohydrate content typical of a low fat diet It is known that a large proportion of ingested glucose is transformed into fat before utilization and since the storage capacity of the body for carbohydrate is limited conversion of carbohydrate into fat is to be expected when food intake exceeds energy requirements (11)

The possibility that low fat diets may be harmful has been discussed

A number of objections may be raised to many of the investigations cited above

1 Some of the studies have been performed on patients rather than normal individuals and since a current view on

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hyperlipemia is that one form is 'carbohydrate induced' due to a disturbance of metabolism not all the studies may be applicable to normal individuals. However, some studies have demonstrated the serum triglyceride raising effect of a low fat diet in healthy subjects (17, 22)

2 In some of these studies in healthy subjects, extremely low intakes of fat have been used in the experimental diet. The result may not be of practical importance since extreme restrictions in fat intake in dietary prophylaxis would not be possible on a large scale. The effect of moderate restrictions is less known, although some studies relevant to this problem have been performed (12-15)

3 Most of the studies have been carried out over a short period of time and have not considered the possibility of habituation. Subjects subsisting on a high carbohydrate diet under poor socio economic conditions do not develop hypertriglyceridemia (3, 10, 18, 19-37). This may be due to a low calory intake or to habituation. Experimental studies in prisoners in South Africa have suggested that the increase in serum triglycerides observed on a high carbohydrate low fat diet (15 per cent of calories) is only temporary, the concentrations returning to base levels after 3—6 months (4). The effect of adaptation has also been observed by other authors in a small number of patients in metabolic ward studies (6), as well as in normal individuals (15)

A previous study in Norwegian lacto vegetarians revealed large, individual variations in fat and calorie intake, with an average intake moderately low in fat and relatively high in carbohydrates (13). The present paper reports a study of triglyceride levels in a similar group having the following characteristics

1 The study included only healthy subjects

2 The diets of the vegetarian participants were only moderately restricted in fat

3 All the participants had lived on the same diet for years

The results have been compared with those obtained in a group of subjects on ordinary Norwegian diet

Determinations of total cholesterol esterified cholesterol, and free fatty acids have been included in the study

Material and methods

The lacto vegetarian group in the study included 31 men aged 40 to 70 years. All had been vegetarians for a number of years, and most of them had participated in a similar study 6 years previously (13)

The control material was made up of 31 employees and workers in the same age group chosen from the files of the industrial physician of a factory in Oslo, Norway. The group was mainly selected from relatively thin individuals to avoid large differences in weight/height relationship between the vegetarian and the control group

The control group had for years had annual health examinations with normal findings. At the time when blood for lipid analyses was drawn, both groups went through an examination including family history, personal history, physical examination, blood pressure reading, urine analysis, sedimentation rate ad modum Westergren, hemoglobin determination, measurements of height and weight, and recording of an electrocardiogram with standard leads and unipolar precordial and extremity leads

In both groups, a family history of diabetes

TABLE I Serum lipids in vegetarians and control material

	Vegetarians				Control material			
	Mean	S D	Median	Range	Mean	S D	Median	Range
Age (years)	51.6	9.1	49	40–70	5[illegible].3	6.8	52	40–67
Weight/height (relationship)	−7.7	6.4	−8.0	−20–+20	−4.9	6.2	−4.0	−18–+10
Total cholesterol (mg/100 ml)	198	30	198	135–[illegible]80	260	45	[illegible]55	163–363
Esterified cholesterol (mg/100 ml)	151	27	152	95–2[illegible]3	19[illegible]	35	189	130–298
Free fatty acids (μ Eq/l)	566	219	550	197–1 035	513	287	460	184–1 478
Triglycerides (mg/100 ml)	79	51	65	16–261	94	40	8[illegible]	30–208

or xanthomatosis a personal history or signs of any major disease a blood pressure higher than 140/90 a sedimentation rate higher than 15 mm in one hour pathologic changes in the electrocardiogram a history of recent weight changes or abuse of alcohol were considered reasons for exclusion from the study All subjects in both groups declared themselves to be in good health and without recent illness of any kind The hemoglobin values ranged from 12.1 to 16.4 g/100 ml in the control group from 11.9 to 14.9 in the vegetarian group

To avoid a bias due to seasonal variations of serum lipids efforts were made to get an equal distribution of subjects from the two groups throughout the period of study

Blood for lipid analyses was drawn following a 12 hour fast and before smoking in the morning The heparin plasma was centrifuged and frozen as soon as possible following venepuncture The lipid analyses were performed in triplicate and the average values are recorded

Free fatty acids were determined according to Dole's method as modified by Trout et al (21 The determination of total and esterified cholesterol have been done with the method of Webster 3 The procedure for triglyceride determination was a modification of the method described by Carlson and Wadstrom

The analytical error for the determination of serum lipids has been calculated by analyses of variance from the triplicate values in the control material The standard errors of observation for total cholesterol esterified cholesterol triglycerides and free fatty acids were 3.5 3.3 4.7 mg/100 ml and 16 μ Eq/l respectively

A dietary survey with an attempt to obtain quantitative estimates was performed in the vegetarian group A dietary history was taken by interview at the same time as blood for lipid analyses was drawn The participants were given a questionnaire with a request to record the quality and quantity of their diet for at least one week The methods used for the dietary survey have been described previously (13 The final calculation of the diet was based on the interview and the questionnaire The calculations included total calories proteins fat carbohydrates saturated fatty acids mono-unsaturated fatty acids and polyunsaturated fatty acids

Attempts were made to perform a similar dietary survey in the control group Due to a greater day-to-day variation in the diet and to lack of food-consciousness of the participants the quantitative information obtained was not suited for further calculations However the survey showed that none of the subjects in this group had peculiar dietary habits

TABLE II Social standing

Social group	Vegetarians	Control group
Independent business	5	
Employees	19	17
Foremen and workers	7	14

Results

The two groups of the study appear comparable with regard to age, the averages being 51 6 and 52 3 years respectively (table I)

A tendency for the vegetarians to have lower weight is apparent from the weight/height relationship evaluated with Broca s formula (weight kg — (height, cm — 100)) with means of — 7 7 and — 4 9 respectively (table I) Two participants one in each group, may be classified as obese with weight/height relationships higher than + 5

The proportion of smokers was higher in the control group (15/31) than in the vegetarian group (5 31)

The social standing of the participants is recorded in table II A small difference is apparent with a higher number of subjects in independent business and a smaller number of foremen and workers in the vegetarian group

The lipid studies table I reveal higher total plasma cholesterol in the control group than in the vegetarians, with means of 260 and 193 mg 100 ml respectively The difference between these means is statistically very highly significant when evaluated by a t test ($t = 6\ 45$, $p < 0\ 001$) The variation of plasma cholesterol is also higher in the control group, with an F ratio of 2 20 between variances ($p < 0\ 05$)

A similar difference is apparent in esterified cholesterol, with mean values of 192 and 151 mg/100 ml respectively ($t = 5\ 16$, $p < 0\ 001$) However, the proportion of esterified to total cholesterol is approximately the same in the two groups, with 76 per cent of esterified cholesterol in vegetarians and 74 per cent in the control group

The triglyceride values are also somewhat lower in the vegetarian than in the control group, with mean values of 79 and 94 mg/100 ml The median values, 65 and 82 mg/100 ml, indicate a distribution of values which does not satisfy the usual requirements for a t test A non parametric test, Wilcoxon s rank sum test, shows that the difference between vegetarians and control material is statistically significant (rank sum vegetarians 816 5, $p < 0\ 05$)

The determinations of free fatty acids did not reveal statistically significant differences between the groups However, values are slightly lower in the control group, and the result of a rank sum test is close to significance (rank sum control material 855, $0\ 10 > p > 0\ 05$)

The means, standard deviations medians and ranges from the dietary survey are recorded in table III

The main characteristics of the diet of the vegetarian participants of the study appear to be the same as found previously in a similar (partly the same) group (13) The diet is in comparison with an ordinary Norwegian diet, mod

TABLE III Dietary survey in vegetarians intake per day

	Mean	S D	Median	Range
Total calories	2 513	656	2 324	1,655–4 009
Protein (g)	69	22	64	30–108
Protein (%)	11	2	11	9–17
Fat (g)	94	39	80	41–205
Fat (%)	33	7	32	20–46
Carbohydrate (g)	347	80	343	208–546
Carbohydrate (%)	56	7	57	43–70
Saturated fatty acids (g)	37	21	32	9–89
Saturated fatty acids (%)	13	5	12	4–22
Monounsaturated fatty acids (g)	29	13	26	7–63
Monounsaturated fatty acids (%)	10	3	10	4–19
Polyunsaturated fatty acids (g)	24	11	20	9–56
Polyunsaturated fatty acids (%)	9	3	8	4–15

erately low in fat (33 per cent of calories) and relatively high in carbohydrates (56 per cent of calories)

The main sources of fat are milk, milk products, vegetable margarines, vegetable oils and nuts. The diet is rich in polyunsaturated fatty acids (25 per cent of fat calories).

The carbohydrates consumed by the vegetarians are mainly derived from bread and cereals, milk, potatoes, fruits and vegetables. From table IV, where the intake of various groups of foods in per cent of calories is recorded together with the consumption in Norway as a whole (41), it appears that the intake of simple sugars with a mean of 4.7 per cent of calories is very much lower than in an ordinary diet. Many of the vegetarians in the study omit ordinary sugar completely from their food. To some extent the difference in intake of simple sugars is reduced by a high consumption

TABLE IV The food composition of the vegetarian diet compared with the total consumption in Norway 1964/65. Per cent of calories from different food resources

	Vegetarians	Total consumption in Norway
Bread and cereals	27.8	25.1
Potatoes	9.8	6.7
Sugar, honey, treacle	4.7	14.4
Vegetables	7.1	0.7
Fruit and berries	10.9	2.9
Vegetable margarines	7.5	5.2
Vegetable oils	7.0	
Other vegetable foods (nuts, peas, cacao, for the vegetarians mainly nuts)	5.0	1.5
Milk and milk products	19	21.0
Animal margarines	0	10.5
Other animal foods (for the vegetarians cod liver oil and eggs for mayonnaise)	1.0	12.0

of fruits, which contain simple sugars making up 60—80 per cent of caloric values. However, even when allowances are made for the high content of glucose, fructose, or sucrose in fruits, the high intake of potatoes and vegetables in the vegetarian group indicates that the proportion of simple sugars to starch and other complex carbohydrates is lower than in an ordinary Norwegian diet.

If individual values for triglycerides in plasma and for dietary intake of fat are considered in vegetarians, a tendency for those having the largest intake of fat also to have the highest triglyceride values is apparent. A calculation of the correlation coefficient between triglyceride values and total intake of fat in per cent of calories shows that this tendency is a statistically highly significant correlation (correlation coefficient = 0.4506, $p < 0.01$).

On the other hand, calculation of the correlation coefficient between triglycerides in plasma and total intake of carbohydrates in per cent of calories reveals a significant negative correlation (correlation coefficient = −0.4015, $p < 0.05$).

Correlation coefficients between triglycerides in plasma and dietary fatty acids in per cent of calories reveal a highly significant correlation between the triglycerides and the sum of saturated and mono-unsaturated fatty acids (correlation coefficient = 0.4855, $p < 0.01$), while the calculations do not indicate any correlation between triglycerides and polyunsaturated fatty acids (correlation coefficient = 0.0406, $p < 0.10$).

Comments

The comparability of a Norwegian vegetarian group and a control material in respects other than dietary habits has been discussed in a previous publication (13). The main difference which possibly affects serum lipids is that of smoking habits. Both in the present and in the previous study the proportion of smokers was higher in the control than in the vegetarian material. This may have affected serum lipid values, but probably not to any great degree. In the present control material, no difference was observed between smokers and non-smokers (median for total cholesterol 256 and 252 mg/100 ml, for smokers and non-smokers respectively, median for triglycerides 86 and 81 mg/100 ml, in the two groups).

In the tabulation of social standing (table II) there is a higher number of men in independent business and a smaller number of workers in the vegetarian group. This would indicate a higher social standing. It is very unlikely that this difference has contributed to the lower serum lipid levels of this group.

In the study, statistically significant differences between the two groups are found in the values for total and esterified cholesterol. Thus, the study has confirmed previous findings regarding total cholesterol values in Norwegian vegetarians (13), and has revealed a proportion of esterified to total cholesterol identical in the two groups. No statistically significant difference was observed between the two groups in free fatty acid values.

The triglyceride values in plasma of the vegetarians have appeared significantly lower than in the control group. This is in disagreement with the findings of Walden et al. among Seventh Day Adventists in New York (22). The group studied by these authors was mainly, but not entirely, consuming a lacto-ovo vegetarian diet. Dietary studies indicated that their diet contained on the average 2,700 calories, with 30 per cent as fat, 15 per cent as proteins, and 55 per cent as carbohydrates. A control group of New York adults on an ordinary diet consumed 3,000 calories, with 41 per cent as fat, 13 per cent as protein, and 46 per cent as carbohydrates. The Seventh Day Adventists were further characterized by a high content of polyunsaturated fatty acids in their diet. Although protein intake indicates that this group is not entirely comparable to the Norwegian vegetarians in dietary habits, they seem to be roughly comparable with regard to fat, carbohydrate and calory intake. On this diet men over 39 years of age in the Seventh Day Adventist group in New York revealed triglyceride values higher than those of New York City adults in general. The authors state that the relatively high carbohydrate, low fat diet of the Adventist men was of great importance in explaining the difference.

In the present study vegetarians on a nearly similar fat and carbohydrate intake revealed triglyceride values which were lower than those of subjects on an ordinary diet. These findings are more in agreement with experimental studies in South African prisoners where a diet containing 3,000 calories, 15 per cent as fat and 70 per cent as carbohydrates, effected only a temporary increase in triglyceride values (4, 5). In fact, after habituation to a low fat diet, the consumption of a high fat diet rich in mono-unsaturated and saturated fats resulted in a significant rise in previously low serum triglyceride values. A high fat diet rich in unsaturated fatty acids kept the levels low.

In the present vegetarian material, there is also a tendency for those with the highest intake of saturated and mono-unsaturated fatty acids to have higher triglyceride values. Obviously, the semi-quantitative estimation of diet is not very suitable for calculations based on the individual values. However, it should be emphasized that the vegetarians are "food-conscious", and have very small day-to-day variations in their food intake, which makes a dietary survey simpler and probably more dependable than in subjects on an ordinary diet. In spite of the objections which may be raised regarding the dietary investigation in the study, the relatively high correlation coefficient between triglycerides in the serum and intake of saturated plus mono-unsaturated fat makes a real relationship probable.

Antonis and Bersohn have suggested that habituation to diets rich in unsaturated fatty acids may lead to increased rates of triglyceride removal from the blood stream, resulting in lower residual triglyceride values. Conversely, habituation to high saturated fat diets may decrease fat-clearance rates, the net result being elevated residual serum triglyceride levels (4, 5). The authors found support for this concept in own

studies, which showed that Bantu subjects had lower levels of plasma radio activity following ^{131}I triolein ingestion than white subjects Since absorption rates appeared to be similar in all the subjects it was inferred that the rates of removal of fat from the plasma was different (5)

The most important contribution of the South African work may be the concept of habituation, which should be remembered when effects of short terme experiments (weeks to months) with dietary influences on serum triglycerides are evaluated

One factor in the diet of the Norwegian vegetarians which may also be of importance is the low sugar content, the high carbohydrate diet being mainly composed of starch and other complex carbohydrates

The different effects of various carbohydrates on serum cholesterol and other lipid fractions have been demonstrated by a number of authors Recently, the effects of starch versus sugar on blood triglycerides have been studied in hypertriglyceremic or hypercholesteremic individuals (14) as well as in healthy males (16 The serum triglycerides rose with a high sugar diet and fell during starch diet periods In both studies changes in the fatty acid composition of plasma were also noted but only a small number of subjects were studied It was concluded that the sugar induced hypertriglyceridemia was caused by active endogenous lipogenesis (14) The mechanism for this phenomenon is unknown but the relatively slow conversion of starch into glucose during digestion and absorption, preventing 'flooding', has been proposed as a possible explanation (14) Some authors have particularly emphasized the importance of the different response of the carbohydrate metabolism to sucrose and to starch loads in this respect (8, 20) An alternative hypothesis is that fructose, a constituent of sucrose but not of starch, is the agent responsible for stimulating endogenous lipogenesis (14) Some authors have demonstrated higher lipid values on sucrose than on glucose in the diet (2, 9, 24)

The triglyceride levels in serum, therefore, may be regulated by a number of factors, including type and proportion of dietary fat (saturated versus polyunsaturated fatty acids) and type and proportion of dietary carbohydrate (sucrose versus starch) Even if these factors operate independently, they may strengthen or oppose each other in their effect on triglycerides, which may explain the varying results regarding dietary regulation of triglycerides

If the type of carbohydrate really is of importance in this respect, it cannot be excluded that the lower proportion of simple sugars to starch and other complex carbohydrates in the vegetarian diet may have contributed to the relatively low triglyceride values in the present study

The high incidence of atherosclerotic disease in the western world calls for prophylactic measures The coming into prominence of very many factors of possible importance in etiology and pathogenesis has greatly confused the issue of prophylaxis However, the dietary changes which have usually been advocated are a moderate lowering of fat intake and some substitution of poly

unsaturated for saturated fatty acids The present study shows that a considerable reduction of plasma cholesterol can be obtained with a diet of this type, without a concurrent increase in plasma triglycerides

Summary

Studies in triglycerides, free fatty acids, esterified and total cholesterol in plasma have been performed in 31 healthy lacto vegetarian males aged 40 to 70 years and in a control group of men in the same age group

A dietary survey showed that the diet of the vegetarians was moderately low in fat (33 per cent of calories) and relatively high in carbohydrates (56 per cent of calories) The intake of polyunsaturated fatty acids was high (25 per cent of fat calories) The intake of carbohydrates was characterized by a lower proportion of simple sugars to starch and other complex carbohydrates when compared with the ordinary Norwegian diet

Large and statistically very highly significant differences were observed in total and esterified cholesterol, with mean values of 198 and 151 mg/100 ml respectively for vegetarians and 260 and 192 mg/100 ml, respectively, for the controls No *statistically significant* difference in free fatty acids was observed between the two groups

Triglycerides were lower in the vegetarian group with median values of 65 and 82 mg 100 in the two groups A non parametric test showed that the difference was statistically significant Calculation of coefficients of correlation between triglycerides in plasma and total intake of fat and carbohydrates indicated that there was a statistically significant tendency for those having the highest triglyceride values also to have the highest intake of fat and the lowest intake of carbohydrates The correlation coefficient between plasma triglycerides and intake of saturated plus mono unsaturated fatty acids was statistically highly significant

It is concluded that a reduction of cholesterol in plasma without a concurrent increase in triglycerides can be obtained with a moderately low fat diet rich in poly unsaturated fatty acids

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From the Institute for Experimental Medical Re[illegible] Head F[illegible]
of Oslo and the Medical Department VII Head [illegible]
Norway

Free Fatty Acids Following Glucose [illegible] Post-gastrectomy Patients

By

KNUT KIRKEBY, KÅRE OVERSKEID and [illegible]

Carbohydrate metabolism is an important regulator of free fatty acid (FFA) concentrations in blood. The administration of glucose as well as of insulin suppresses the release of FFA and lead to a decrease in plasma levels (2, 6).

Disturbances in carbohydrate and lipid metabolism can often be demonstrated in patients who have undergone gastric surgery. In patients with a partial gastrectomy and a gastro-jejunal anastomosis a rapid dumping of and absorption of carbohydrates may result in hyperglycemia, an exaggerated insulin response and subsequently hypoglycemia (4). On the other hand the absorption of fat is disturbed in the majority of these patients ([illegible] 10, 12, 14), probably due to an inadequate mixing of the fat meal with bile and pancreatic enzymes.

The present study was undertaken to evaluate the FFA response to a glucose or a fat load in post-gastrectomy patients.

Material and methods

Fi[illegible] post-gastre[illegible] healthy individuals [illegible] were all males and the [illegible] matched for age with the pat[illegible] [illegible] gastrectomy which was a Billroth II procedure had been performed for ulcer at least 3 years prior to the study. All these patients complained of some post-prandial discomfort. With the exception of the ulcer disease and the gastrectomy neither the patients nor the control group had a family or personal history of disease known to affect lipid or carbohydrate metabolism.

Glucose and FFA in plasma were determined following a 12 hour fast and at 1/2, 3/4, 1, 1 1/2, 2, 2 1/2, 5 and 10 hours after the ingestion of 1 g of glucose per kg body weight. The same determinations were made two days later after the ingestion of 1.2 g of olive oil per kg body weight.

In the fat load experiment triglycerides in plasma were also determined fasting and at 1, 2, 5 and 10 hours after the fat intake.

Plasma glucose was determined by a glucose-oxidase method (3) and FFA according to Dole's method as modified by Trout et al. (2, 13). The procedure for triglyceride determination was a modification

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TABLE I FFA and glucose in plasma following a glucose or a fat load in post gastrectomy patients and in controls Mean values with standard error of the mean in brackets

	FFA in plasma (µequiv /liter)		Glucose in plasma (mg/100 ml)	
Hours	Post gastrectomy group	Control group	Post gastrectomy group	Control group
Glucose load 1 g/kg body weight				
0	395 (36)	405 (99)	96 (4)	94 (4)
1/2	210 (37)	315 (77)	155 (21)	145 (10)
3/4	185 (30)	283 (71)	161 (20)	138 (13)
1	197 (45)	263 (70)	160 (17)	124 (10)
1 1/2	191 (34)	232 (59)	108 (11)	105 (10)
2	228 (43)	198 (42)	76 (13)	95 (11)
2 1/2	375 (62)	391 (63)	72 (10)	92 (10)
5	493 (54)	518 (110)	92 (5)	90 (5)
10	673 (118)	694 (92)	95 (8)	93 (5)
Olive oil load 1/2 g/kg body weight				
0	396 (43)	301 (46)	97 (6)	93 (4)
1/2	401 (32)	334 (68)	93 (4)	88 (4)
3/4	385 (36)	351 (55)	97 (6)	86 (5)
1	370 (46)	331 (71)	91 (4)	87 (6)
1 1/2	357 (69)	284 (40)	94 (4)	89 (5)
2	461 (73)	402 (53)	98 (4)	88 (5)
2 1/2	442 (29)	444 (75)	100 (5)	88 (6)
5	563 (31)	486 (45)	105 (4)	87 (6)
10	647 (50)	545 (59)	98 (4)	85 (6)

TABLE II Triglycerides in plasma following a fat load (1/2 g/kg body weight) in post gastrectomy patients and controls Mean values with standard error of the mean in brackets mg/100 ml

Hours	Post gastrectomy group	Control group
0	83 (11)	97 (13)
1	81 (10)	109 (14)
2	107 (12)	130 (15)
5	109 (15)	132 (13)
10	85 (12)	99 (13)

of the method described by Carlson and Wadstrom (1) All analyses were performed in duplicate (glucose) or triplicate (FFA and triglycerides) and the average values are presented

Results

The results are recorded as mean values with standard error of the mean in brackets in tables I and II

A graphical presentation of the mean values of glucose and FFA following a glucose load in the two groups is given

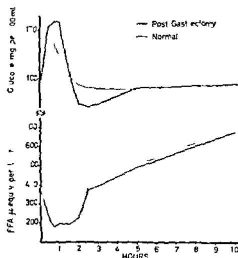


Fig 1 FFA and glucose in plasma following a glucose load Mean values

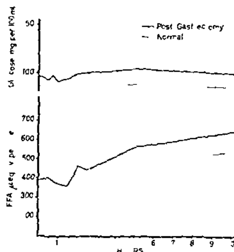


Fig 2 FFA and glucose in plasma following a fat load Mean values

in *fig 1* The glucose tolerance curves show that the response to a glucose load is greater in the post gastrectomy group than in the control patients, with higher glucose values at 1/2 to 1 hour after the administration of glucose and with a tendency to post hyperglycemic hypoglycemia Three of the five post gastrectomy patients, but none in the control group had glucose levels below 65 mg/100 ml 2 to 1 1/2 hours following the intake of glucose

Some difference in the pattern of FFA response to the glucose load is noted Lower values are found in the post gastrectomy group at 1 2 to 1 hours following the intake of glucose The difference is particularly great 1/2 hour after the glucose administration with mean values of 315 and 210 μ equiv liter respectively in the two groups However the subsequent rise in FFA concentrations appears similar in the two groups and during the fasting period from 2 1/2 to 10 hours all participants showed a gradual increase in values

The patterns of glucose values following a fat load do not reveal changes during the 10 hour period of study and do not reveal differences between the two groups (fig 2)

The changes in FFA following a fat load are small and unimportant during the first two hours in both groups (fig 2) and subsequently a similar gradual increase during the fasting period as in the experiments with glucose load is observed

The triglycerides in plasma following the fat load revealed an increase with a peak at 5 hours in both groups (table II) The increment in mg 100 ml was higher in the control group than in the post gastrectomy group particularly at one hour after the fat intake

TABLE I FFA and glucose in plasma following a glucose or a fat load in post gastrectomy patients and in controls Mean values with standard error of the mean in brackets

Hours	FFA in plasma (μequiv/liter) Post gastrectomy group	Control group	Glucose in plasma (mg/100 ml) Post gastrectomy group	Control group
Glucose load 1 g/kg body weight				
0	395 (36)	405 ([illegible]9)	96 (4)	94 (4)
1/2	210 (37)	315 ([illegible]7)	155 (21)	145 (10)
3/4	185 (30)	283 ([illegible]1)	161 (20)	138 (13)
1	197 (45)	263 (70)	160 (17)	124 (10)
1 1/2	191 (34)	232 (59)	108 (11)	105 (10)
2	228 (43)	198 (42)	76 (13)	95 (11)
2 1/2	375 (6[illegible])	3[illegible]1 (63)	72 (10)	92 (10)
5	493 (54)	[illegible]18 (110)	92 (5)	90 (5)
10	673 (118)	694 (92)	95 (8)	93 (5)
Olive oil load 1/2 g/kg body weight				
0	396 (43)	301 (46)	97 (6)	93 (4)
1/2	401 (32)	334 (68)	93 (4)	88 (4)
3/4	385 (36)	351 (55)	97 (6)	86 (5)
1	3[illegible]0 (46)	331 (71)	91 (4)	87 (6)
1 1/2	357 (69)	284 (40)	94 (4)	83 (5)
2	461 ([illegible]3)	402 (53)	98 (4)	88 (5)
2 1/2	44[illegible] (29)	444 (75)	100 (5)	89 (6)
5	563 (31)	486 (45)	105 (4)	87 (6)
10	647 (50)	545 (59)	98 (4)	85 (6)

TABLE II Triglycerides in plasma following a fat load 1/2 g/kg body weight in post gastrectomy patients and controls Mean values with standard error of the mean in brackets mg/100 ml

Hours	Post-gastrectomy group	Control group
0	83 (11)	97 (13)
1	81 (10)	109 (14)
2	107 (1[illegible])	130 (15)
5	109 (15)	13[illegible] (13)
10	85 (12)	[illegible] (13)

of the method described by Carlson and Wadstrom (1) All analyses were performed in duplicate (glucose) or triplicate (FFA and triglycerides) and the average values are presented

Results

The results are recorded as mean values with standard error of the mean in brackets in tables I and II

A graphical presentation of the mean values of glucose and FFA following a glucose load in the two groups is given

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From the Department of Internal Medicine (Head Nils Tornblom D Umea University Umeå, Sweden

Localization of Plasma Proteins in Small Dermal Blood Vessels in Diabetes

Preliminary Report

By

OLLE LARSSON

Using a fluorescent antibody technic Larsson and Melin (1) in 1964 demonstrated bound ' gamma globulin in the walls of the small blood vessels in the skin from six diabetics but not from two healthy individuals

This investigation has now been extended and includes 82 patients with diabetes and 35 healthy individuals The findings have been correlated to some clinical data and to the histological appearance of the blood vessels with PAS staining

With a similar technic it was investigated whether γA γM β_{1C} globulin fibrinogen and albumin could be identified in the vessel walls together with γG globulin This investigation included 20 diabetics and 11 healthy individuals

Skin was taken by punch biopsy from normal appearing areas of the calf Interest was focused especially on the small blood vessels beneath the epidermis The term small vessels is used since it was not possible in this investigation to distinguish with certainty capillaries from other minor vessels

Both direct and indirect methods of immunofluorescent staining were used to identify γG globulin the other plasma proteins were identified by the indirect method alone The result was rated as (—) if no or only traces of specific fluorescence could be seen as (+) if the fluorescence was moderate but distinct and as [illegible] as strong and bright

In positive cases [illegible] localized in the basement membrane region as a single [illegible] multiple band [illegible] the endothelial cells [illegible] fluoresced Sometimes a rather [illegible] fluorescence was noted subend[illegible] in the whole vessel wall Both positive and negative vessels were usually present in a given section Sometimes only part of a wall fluoresced The positive picture was similar for all other investigated proteins except albumin Here the fluorescence often was seen more peripherally and was more diffusely demarcated

In the PAS-stained sections the basement membrane of the vessels was estimated as normal grade I or moderately (grade II or strongly grade III) thickened

The preliminary results are presented in tables I IV

No positive correlation was seen between the age of the patients and the result of the immunofluorescent staining

Positive staining for γG globulin was seen in 20 (57 %) of 35 diabetics without

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TABLE I Localization of γG-globulin in the walls of small dermal vessels

	No	Age	Median age	γG-globulin (+)	(++)	(%)
Diabetes	82	8–80	41	24	31	67
Healthy	35	16–74	33	6	1	20

TABLE II Diabetes. Localization of γG-globulin in small dermal vessels related to the basement membrane thickness (grade I—III)

γG globulin	(−)			(+)			(++)		
Grade	I	II	III	I	II	III	I	II	III
Number	12	11	7	3	12	9	1	7	23

TABLE III Diabetes. Localization of γG-globulin in the walls of small dermal vessels related to known duration of the disease

Duration (yrs)	0–5	6–10	11–15	16–
No investigated	28	14	18	22
Positive (%)	46	57	67	86

TABLE IV Localization of plasma proteins in the walls of small dermal vessels

Protein	γG		γA		γM		β1C		Fibrinogen		Albumin	
Fluorescence	+	++	+	++	+	++	+	++	+	++	+	++
Diabetes	6	7	4	1		1	2	1	4	2	3	0
(20 pats)	65%		25		30		15%		30%		15%	
Healthy	4	0	1	0	0	0	0	0	0	0	2	0
(11 pats)	36%		9%		0%		0%		0%		19%	

signs of retinopathy, nephropathy, neuropathy or dermopathy. This was also seen in 11 (44%) of 25 patients who had not been treated with insulin.

Summary

In diabetes immunoglobulins, β_{1C} globulin, and fibrinogen were demonstrated in the walls of small dermal blood vessels in a pattern that was different from that seen in healthy individuals. The results are correlated with the duration of the disease but not with the age of the patients.

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